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THE QUALITATIVE NATURE OF PULSATILE FLOW IN UMBILICAL BLOOD VESSELS, WITH OBSERVATIONS ON FLOW IN THE AORTA*

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The results reported in this paper arise from two lines of interest. One is the relationship of the mechanism of venous return in the umbilical vein to the rest of the fetal circulation; the other, the use of the umbilical blood vessels, especially the arteries, to determine some of the basic characteristics of pulsatile flow in elastic tubes.

With respect to flow in the umbilical vein, *a priori* considerations suggest that the high pressure in the distended umbilical vein and the physical relation of this vessel to the umbilical arteries allow pressure arising from arterial deformation associated with pulsatile flow to aid the flow of blood in the vein. Such deformation would add transient, if slight, pressure to the column of venous blood. Thus the cord as a unit could act like a pulsameter pump in facilitating to a degree the flow of blood from the placenta to the fetus (Reynolds, 1951).

With respect to the nature of pulsatile flow in blood vessels, there were several questions to which answers were desired. First, is there appreciable loss of energy from frictional resistance to the flow of blood in the artery? Second, to what extent is there lateral deformation, or increase in diameter, of the umbilical and other arteries as the pressure varies with each cardiac ejection of blood into the aorta? Finally, what is the average linear velocity of blood flow in the umbilical artery? It was desirable to know these facts simultaneously with arterial pressure. The umbilical blood vessels are especially useful for the estimation of these factors since the cord is accessible and it affords an extended length without giving rise to major branches. Umbilical cords lend themselves well, therefore, to angiographic studies of the velocity of blood flow and the nature and extent of deformation of the arterial walls during pulsation.

In addition to the foregoing points, observations have been made simulta-

* The authors are indebted to Drs. Geoffrey S. Dawes and Derek G. Wyatt, of the Nuffield Institute for Medical Research for assistance and advice in this entire project, particularly in instrumentation and recording.

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neously on other topics of ancillary interest. These have to do with the characteristics of pulsatile streamlining in arteries, the effect of spiralling of the artery on the movement of the column of blood in the umbilical artery and some aspects of the energy relationships of blood flow.

TABLE I

Breed, weights and ages of fetal lambs

NUMBER	BREED	WEIGHT (Kilos)	AGE		CONDITIONS OF OBSERVATION
			estimated	given	
S 2	Hampshire	3.5	140	—	Direct Cine
S 3	Hampshire	3.81	140	—	Direct Cine
S 5	Hampshire	4.15	145	—	Direct Cine
S 7	Hampshire	4.4	145	—	Direct Cine
S 9	Hampshire	3.8	140	—	Direct Cine
S 11	Hampshire	5.2	147	—	Direct Cine
S 13	Hampshire	3.18	140	—	Indirect cine
S 14	Hampshire	3.15	136	—	Indirect cine
S 17	Welsh	2.75	—	130	Indirect cine
S 18	Welsh	2.75	—	130	Indirect cine
S 20	Welsh	4.70	—	145	Indirect cine
S 21	Welsh	4.70	—	145	Indirect cine; blood pressure
S 23	Welsh	2.3	—	126	Direct cine, 2 frames/sec; blood pressure
S 25	Welsh	2.3	—	136	Indirect cine; blood pressure
S 27	Welsh	2.5	—	133	Indirect cine; blood pressure
S 29	Welsh	2.8	—	136	Indirect cine; blood pressure
S 31	Welsh	2.6	—	136	Blood pressure only
S 33	Welsh	2.8	—	136	Indirect cine; blood pressure
S 35	Welsh	2.1	—	130	Indirect cine; blood pressure
S 37	Welsh	2.6	—	136	Indirect cine; blood pressure
S 39	Welsh	3.3		140	Indirect cine; blood pressure
S 41	Welsh	2.3	—	122	Indirect cine; blood pressure

METHODS

Animals

The observations reported in this paper were made on 22 fetal lambs and 2 adult cats. Details regarding the breed, weight, and age of the lambs are given in Table I.

Operative procedures

The fetal lambs were delivered by Caesarian section of ewes anesthetized with Dial urethane, 0.4 gm. urethane and 0.1 gm. diallylbarbituric acid per cubic centimeter given at 0.7 cubic centimeters per kilogram of body weight (Ardran, Dawes, *et al.* 1952). At the moment of delivery a rubber bag filled with amniotic fluid was secured in position over the head of the fetus to prevent breathing. The placenta was left *in situ* and the umbilical cord protected against undue tension, cooling and drying. Cotton wool and an electric heating pad were used to keep the fetus warm. Cannulae or needles were then inserted into umbilical

or other vessels for injections or blood pressure measurements. If cineangiograms were to be taken the fetus and/or the umbilical cord were placed in position over the appropriate x-ray apparatus.

The cats, which were used for a study of the flow in the aorta, were anesthetized with chloralose (70 mg./kg.). During artificial respiration part of the left side of the chest wall was removed to facilitate radiography of segments of the thoracic aorta, and later the abdomen was opened to expose a portion of the aorta in this region. A cannula was inserted into one of the left pulmonary veins for injections of contrast medium.

Contrast media

Thorotrast (a 25% colloidal preparation of thorium dioxide) was used in most experiments, but in some instances a 35% solution of diodone was employed (Reynolds, Ardran and Prichard).

Apparatus for Angiography

In this work three types of angiographic apparatus were used:

1) An apparatus taking direct serial radiographs (150 x 60 mm.) at a rate of 2 per sec., each with an exposure time of 0.05 sec. This was used in only one experiment (S23.1).

2) A 35 mm. cine camera taking direct radiographs (24 x 18 mm.) at a rate of 25 per second (Ardran and Tuckey, 1952). The duration of exposure of each radiograph, or frame of the cine film, was 0.03 sec. This apparatus was used for observing the circulation in a short segment of the umbilical cord.

3) A 35 mm. cine camera taking indirect radiographs (photofluorograms) at a rate of 25 per sec. (Ardran and Tuckey, 1952). The duration of exposure of each frame of the cine film was 0.03 sec. This was used in the majority of experiments and recorded a fluorescent screen image 240 x 180 mm. or larger.

Both the 35 mm. cine cameras were operated synchronously with a 50 cycle A.C. main supply.

Blood Pressure Recording

In the fetal lambs the blood pressure in individual vessels was measured by means of needles connected to a pair of saline-filled capacitance manometers (Ardran, Dawes *et al.* 1952). The record was displayed and photographed on a cathode-ray oscilloscope, together with time in seconds and (on occasion) the duration of each x-ray exposure. The blood pressure in an artery in the umbilical cord was determined by one of two methods. In the first an 18 or 20 gauge hollow needle was pushed through the wall of the artery. In the second, a cannula was tied in to one of the first branches of the umbilical artery so that the tip of the cannula lay *just within* this branch. In the latter instance the true lateral arterial pressure could be recorded without deformation on the vessel wall or interference with the flow of blood in the main artery. For measuring the blood pressure in one of the veins within the umbilical cord a needle was pushed through the wall of the vessel itself. This did not give an absolute measurement of venous blood pressure, but was considered sufficient for the immediate purpose. In some instances the blood pressure in the distal abdominal aorta was recorded by means of a polythene catheter passed through the femoral artery.

RESULTS

Umbilical artery blood pressures. Attempts to measure blood pressure in the umbilical artery by direct puncture of the main vessel with the recording

needle yielded unsatisfactory results. It was found from the cineangiograms that insertion of the needle into an artery caused complete or partial constriction of the artery. The immediate and severe constriction of the artery on insertion of the needle was evident to the observers. Although a pressure could be recorded at this time angiograms showed that the lumen was obliterated. Later, when it was believed from external observation that the spasm had relaxed and that the flow of blood should now be normal, further angiography showed that the site of the artery through which the needle passed was greatly deformed, and that the stream-line pattern of flow was disturbed in this part of the vessel. It follows, therefore, that although records of umbilical arterial blood pressure which approximate to a normal value may be obtained, it is impossible to record absolute pressures or the true form of the pulse wave by this means. The figures quoted in the literature on this point (Barcroft, 1947; Reynolds, 1951) must be regarded as of limited value.

In order to avoid the artefacts consequent on piercing the vessel wall we inserted a non-bevelled cannula into the first branch of the umbilical artery at a point about one centimeter from the origin of the branch, and then advanced it until the tip lay just within the branch and at right angles to the main artery. With this method the flow in the main umbilical artery was not disturbed and a true record of the lateral pressure was obtained. A graph of the systolic and diastolic levels during some thirty consecutive cardiac cycles is shown in figure 4.

Umbilical arterial and venous pulses. Simultaneous pressures were measured in an umbilical artery and vein under various circumstances. For example in one instance the blood pressure in the artery was 79/72 millimeters of mercury, that in the vein 43/40 millimeters of mercury. The pulsations were not in phase with each other. Repeated observation showed that they were approximately 180° out of phase. That is to say, as the arterial pulse pressure increased, the pulse pressure in the vein diminished, and vice versa. An example of this is shown in the venous and arterial pressure records in figure 1.

Since there are two umbilical veins in the cord of the sheep, temporary interruption of flow down one leaves the other to carry blood back to the fetus. When one vein was occluded between the umbilicus and the point of recording, the pressure in it was still pulsatile. Fig. 1 (middle record) shows that the mean pulse pressure was as much as 1.2 mm. Hg. (average of 52 consecutive pulsations). When the vein was occluded between the placenta and the point of recording, the pressure in it was also still pulsatile (Fig. 1, top record), and still about 180° out of phase. Since pressure pulsation was observed in the umbilical vein when either the umbilical end or the placental end of the vein was occluded, this pulsation may not result wholly from transmitted pulsation along the vein.

Two attempts were made to record the pressure pulse in a static column of

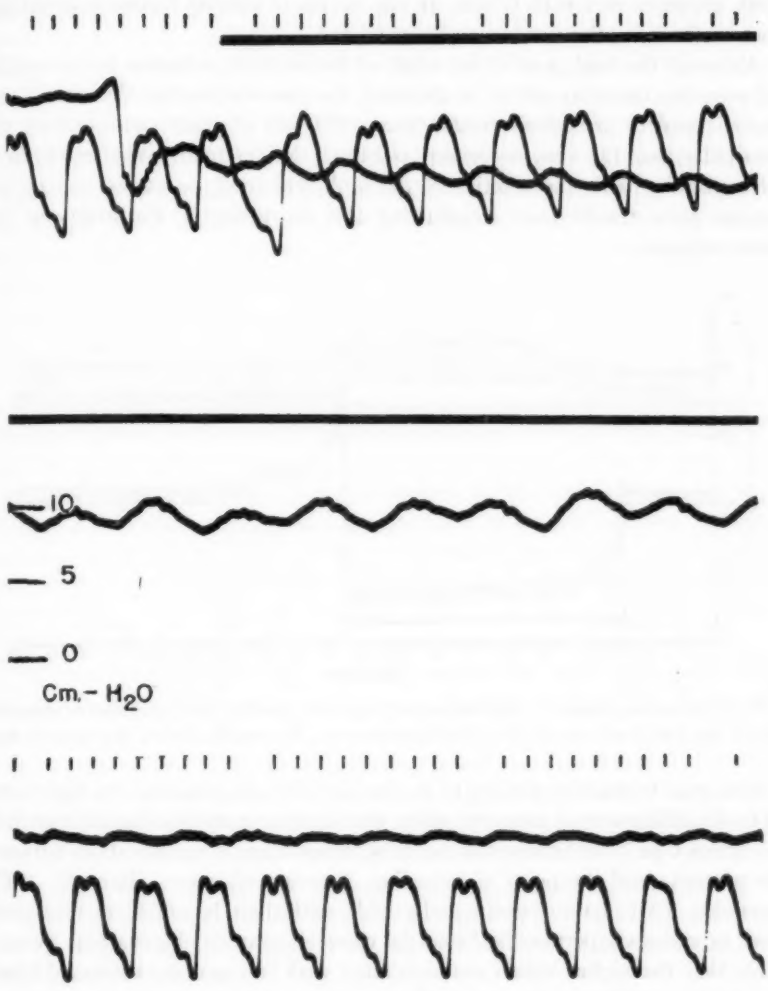

FIG. 1. Arterial and venous pulsations in umbilical vessels. *Bottom*, venous and arterial pulsations with unrestricted blood flow. *Middle*, venous pulsations in a static column of blood resulting from occlusion between the fetus and the recording needle. *Top*, effect of occlusion of placental end of vein on venous pulsations. Time, 0.1 second.

blood in the vein after occlusion of both the fetal and placental ends of the vein. Both attempts met with failure. In the course of passing ligatures about the ends of the vein, the entire vessel constricted.

Although the final proof of the origin of the pressure pulsation in the umbilical veins has therefore still to be obtained, the observation that the arterial and venous pressure pulsations remain about 180° out of phase could exclude the possibility that the venous pressure pulsation derives to any large extent from its contiguity with the arteries of the umbilical cord, unless, of course, the venous pulse results from lengthening and shortening of the artery as the latter pulsates.

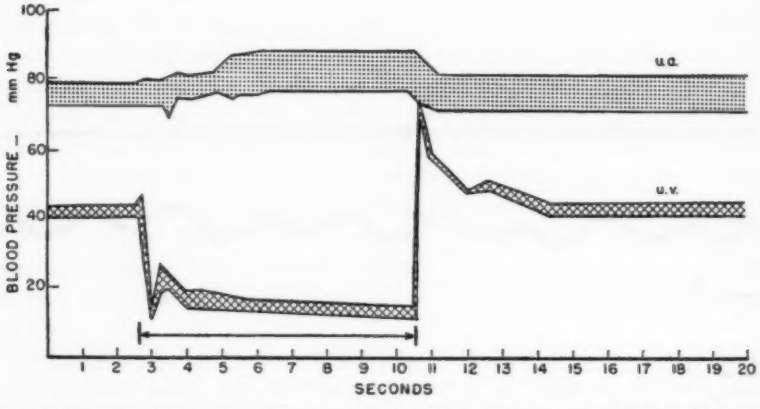

FIG. 2. Pressure changes in umbilical artery and vein resulting from occlusion of placental end of one umbilical vein. U. A., umbilical artery; U. V., umbilical vein. See figure 1, top.

Note may be made in passing of the fact that venous pressures are high (circa 30 to 40 millimeters of mercury) when the blood is freely flowing but that it is low (circa 6 to 10 millimeters of mercury) when there is venous stasis between the placenta and the point of recording. Previous observers (Barcroft, 1947; Reynolds, 1951) have found equally wide variations in umbilical vein pressures in various experiments. From the cineangiographic observations we conclude that the higher values are associated with free and unobstructed blood flow in the umbilical vein, and the lower values with restricted blood flow.

Pressure gradients in the umbilical arteries. Since absolute measurements of lateral pressures could not be obtained in the unbranched portion of the umbilical artery, it was impossible to determine directly the pressure gradient along one umbilical artery. Reynolds (1951) had suggested that there was a pressure gradient of 0.5 to 1.0 mm. Hg. per centimeter of artery, but in view of the present observations on the effects of direct puncture of the umbilical ar-

tery, this view may have to be revised. Certainly, the pressure gradient was insufficient to cause any measurable variation in the diameter of the umbilical artery, as shown by angiograms of the whole vessel during a complete pressure cycle.

The relation of visible pulsation in arteries to the pulse pressure wave

Umbilical arteries. The angiograms demonstrating the circulation through the umbilical cord were examined with a view to determining whether there was any change in the calibre of the umbilical arteries or any bodily displacement of these vessels, which was related to the pulse pressure wave. Except in one instance the angiograms which provided the data on this point were taken by the direct method at a speed of 25 per second, and thus at least 8 angiograms were obtained during a single pulse wave. Measurements were taken both from the films directly and also from enlarged images of the vessels.

In one experiment an obstruction was present in one of the umbilical arteries, and proximal to this obstruction alterations in the calibre of the artery were seen which coincided with the pulse pressure wave. In no other instance, however, was it possible to detect any alteration in the calibre of the umbilical arteries at any phase of the pulse pressure wave. When the positions of selected points along the artery were measured in relation to fixed points on the film, it was found that it was only at the apex of curved portions of the vessel that a movement could be detected. This movement consisted of a bodily displacement of the whole artery of the order of 0.2 mm. (Figs. 3 and 6).

On visual inspection of the umbilical cord, at the time when these angiograms were made, the arteries had been seen pulsating vigorously. The records show that the visual impression is erroneous in that there is no detectable alteration in the calibre of the artery with the pulse pressure wave.

Aorta. In order to determine whether these observations are peculiar to the arteries within the umbilical cord, or whether the principle involved is of more general application, a study was made of the aorta of two adult cats. Direct cineangiographic records were made at a rate of 25 frames per second of four segments of the aorta: the ascending aorta, the arch, the descending thoracic, and the abdominal aorta (Fig. 7). Measurements of the vessel were made from enlargements of the films.

A change in the calibre of the vessel was seen to occur only in that portion of the vessel nearest the heart, i.e., in the ascending aorta and in the arch. Here there was a large increase in the calibre of the vessel with each ejection of blood from the heart, together with a bodily displacement of the whole vessel. No alterations in calibre could be detected in the descending thoracic or abdominal aorta. These observations confirm the findings of Timm (1947–48) who used a somewhat different cineradiographic technique.

Streamline flow in the umbilical artery. Direct cineangiograms showed that when the artery spiralled, the entire column of fluid maintained a streamline flow and spiralled about the long axis of the vessel (Fig. 5).

The basic fact to be noted from this part of the study is the following: When the artery is spiralled, the entire column of fluid within the vessel travels

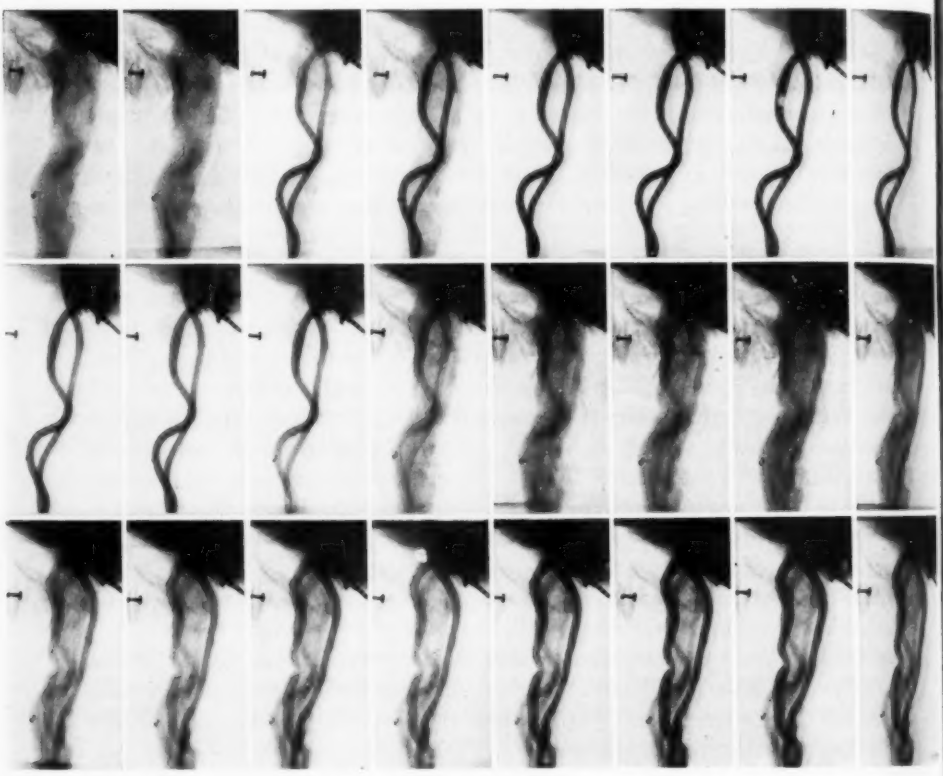

FIG. 3. Photographs of the umbilical cord of the sheep, taken at intervals of one-half second. Thorotrast was injected into the base of the aorta. Five and one-half seconds after beginning to fill the arteries it is seen returning in the veins. See figure 4 and text. Placental end of cord at top of picture.

spirally, too. This means that some of the advancing column is moving a greater distance around the outer curve of the spiral than is the inner part of the column which follows the inside of the spiral. The former is held there by centrifugal force. The outer, moving portion of the column possesses, therefore, greater kinetic energy, and it must tend to remain stabilized in that outer position for that reason.

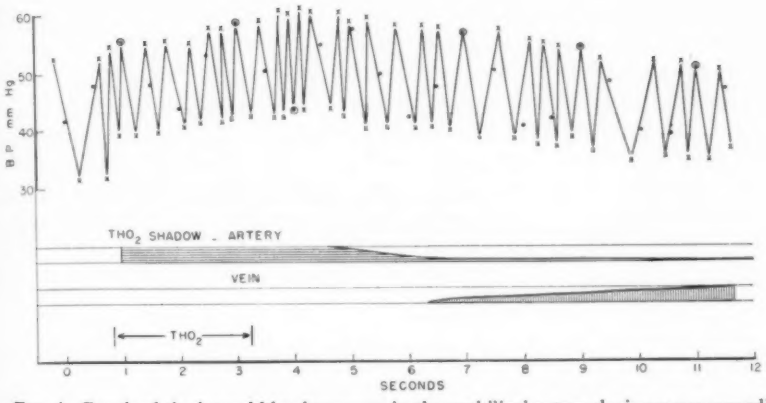

FIG. 4. Graph of the lateral blood pressure in the umbilical artery during x-ray recording of the pictures shown in figure 3. The exposures were made at moments indicated by the dots. Some were at systole, one was at diastole, and others at points in between.

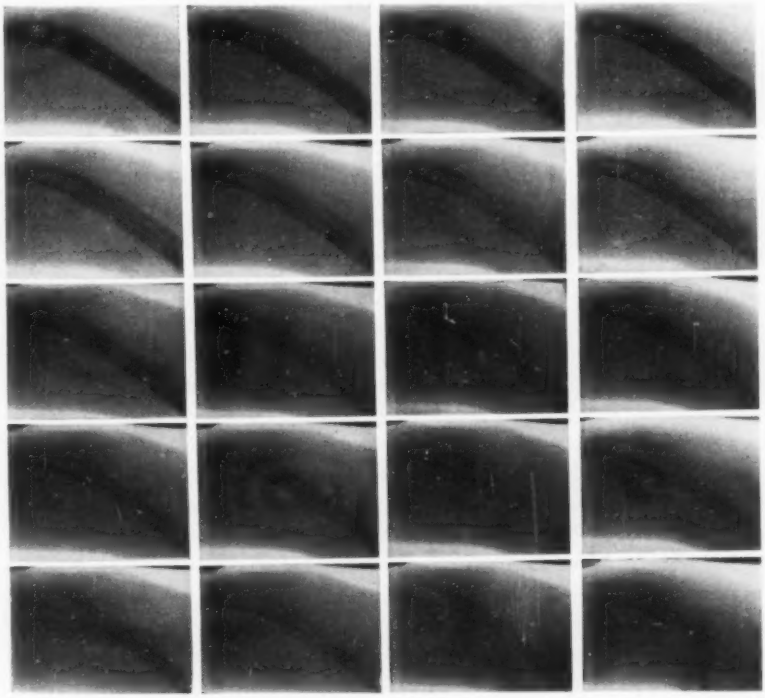

FIG. 5. Twenty consecutive direct cineangiographs in order, reading from left to right. Two cardiac cycles. Note the stream-lines, the absence of change in diameter of the vessel with pulsation, and evidence of pulsatile flow in the shift of the angles of crossing of principal stream-lines. The curve shown is actually a picture of part of a spiral.

When pulsation is superimposed upon this basic form of fluid movement (laminar flow), the quick component of the pulse during the period of increasing pressure is thrust with force momentarily ahead against a more distant point on the outer curvature of the restraining vessel wall. At a point of curvature, the vessel wall will be moved or be displaced slightly. Since the pressure is transient, the vessel recoils to its previous position. In this way the impression of pulsation is gained when one watches the phenomenon of unrestricted pulsatile blood flow in an artery as described in the preceding section. Although this is best seen in cineprojection of these pulsating stream-lines, still pictures of the phenomenon are shown in figure 5.

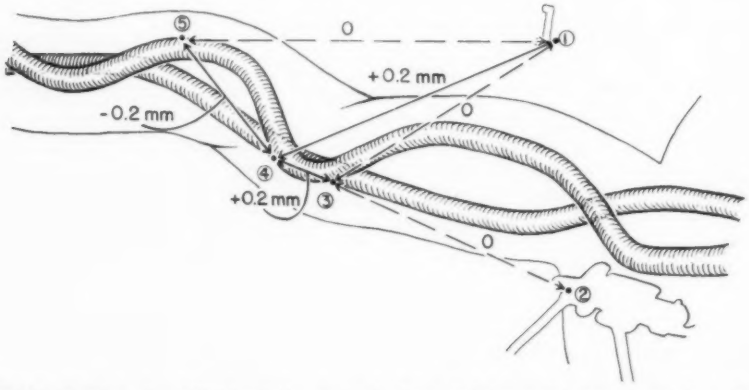

FIG. 6. Outline of umbilical arteries shown in figure 3 (picture 7 taken at systole and picture 9 at diastole) showing maximum shift in arterial positions on the film from systole to diastole relative to two fixed points, 1 and 2.

Blood flow in the aorta and umbilical arteries. Direct measurements of the blood flow in the aorta and umbilical arteries of the fetus are difficult to obtain, but an estimate can be made by cineangiography. A study was made of indirect cineangiograms, taken at a rate of 25 per sec., which showed the circulation through the aorta and umbilical arteries in 5 fetuses, in which similar experimental conditions prevailed. The animals had not breathed and the observations were made in the early part of the experiment.

From the films the linear velocity of the blood flow was calculated by measuring the time taken for the advancing head of the contrast medium to pass between two selected points of the vessels in question. The diameters of the lumina were measured and an estimate made of the distances traversed. The calculations are summarized in Table II.

Direct cineangiograms of 5 further fetuses, demonstrating the circulation through a short length of umbilical cord only, provided additional data on the

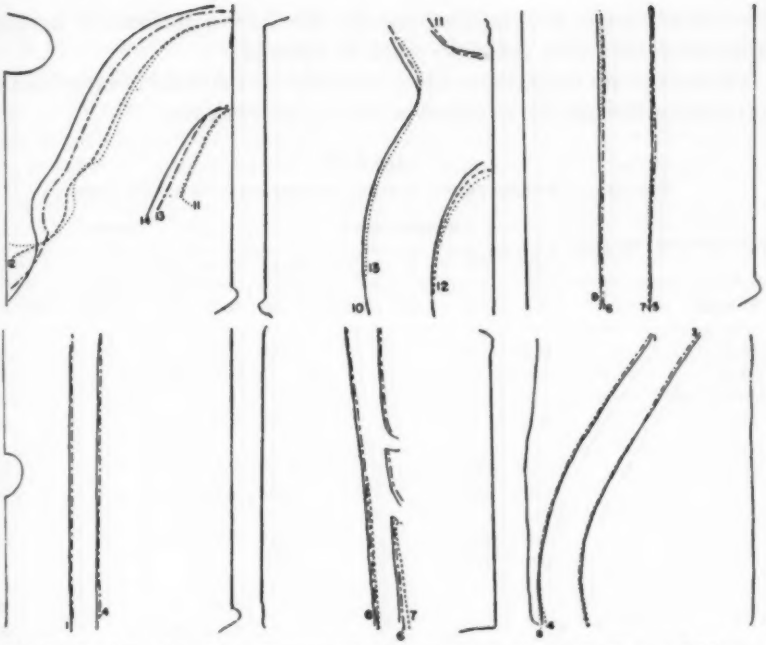

FIG. 7. Tracings of outlines of consecutive frames of arterial walls taken at 0.04 second intervals on 35 millimeter film. From top left, as follows: ascending aorta and arch; arch and beginning of descending aorta; thoracic descending aorta. Bottom, from left: upper abdominal aorta; lower abdominal aorta (all of the adult cat); lamb umbilical artery.

The frames traced were chosen to show the maximum distention or displacement seen in a series of twenty frames (i.e., two to three heart beats) at each level of the artery. Parallel lines at the sides indicate the edges of each frame. They were 18 millimeters apart on the original film.

TABLE II

Blood-flow rates and volumes in the umbilical cord and aorta

FETUS	WEIGHT	FETAL AGE x/147 DAYS	UMBILICAL ARTERY				AORTA FLOW PER MIN.	VELOCITY CMS/SEC IN AORTA	UMBIL. ARTERY AORTA RATIO
			Velocity	Area artery	Flow per min., 2 arteries	Unit of flow / flow per kg/min			
	Kg		*cms/sec.*	*cm²*	*ccs.*	*ccs.*	*ccs.*		%
S 27	2.5	133/147	83	0.07	696	278	933	55	75
S 29	2.8	136/147	100	0.05	636	224	990	33	64
S 25	2.3	136/147	40	0.16	768	334	1411	83	54
S 21	4.7	147/147	166	0.09	1788	380	2820	80	63
S 20	4.7	147/147	143	0.12	2040	434	3280	60	60
Aver.									63.3

blood flow through the umbilical arteries. The figures obtained in the latter experiments fell within the range noted in Table II.

The estimations suggest that about two-thirds of the blood flowing down the aorta passes through the umbilical arteries to the placenta.

TABLE III

Estimation of Reynolds number in umbilical artery and aorta in lamb fetuses

FETUS OR TWO-DAY OLD LAMBS	UMBILICAL ARTERY					AORTA				
	Diam.	Vel.	ρ	μ*	Re no.	Diam.	Vel.	ρ	μ	Re no.
S 27 fetal	0.3	83	1.027	0.022	1140	0.55	55	1.027	0.022	1385
S 29.1	0.25	100			1140	0.8	33			1240
29.3						0.8	48			1792
S 25	0.45	40			850	0.6	83			2300
S 21	0.35	166			2712	0.95	72			3160
	0.35	180			2940	0.95	80			3542
S 20	0.4	143			2630	1.0	60			2770
	0.4	111			2072					
S 37						0.72	25			840
						0.72	30			1009
S 13						1.0	25			1149
						1.0	16.6			779
S 35						0.6	36			1109
S 41						0.45	33			700
Goat fetus, G 4						0.65	30			910
Two-day old lambs, S 42										
chest closed:						0.8	33			1232
chest open:						0.78	18			653
S 43						0.77	66			2371

* Based in c.g.s. units for water at 10°C = 0.0131 and specific viscosity of 1.7 for blood.

Energetics of Flow (*by F. W. Light, Jr.*)

Streamline flow and the velocity head. The observations on flow down the umbilical arteries provide data for certain other calculations to be made.

The form of the streamlines in a moving fluid, and whether or not they exist, is governed by the Reynolds number, which for a circular tube is

$$\mathrm{Re} = \frac{\rho D V}{\mu}$$

where ρ is the density of the fluid, μ its coefficient of viscosity, V its velocity, and D the diameter of the tube. When V exceeds a certain amount, other conditions remaining constant, the flow becomes turbulent, and this happens when Re is greater than about 2100. The exact critical value of Re depends on a variety of factors, such as curvature of the tube, its relative roughness, and flow conditions at the inlet. Also, it is to be noted that blood is not a true liquid.

A curvature in a pipe carrying water raises the threshold for the onset of turbulence from about 2000 to over 7000 (Macadams, 1933). The critical Reynolds number likewise varies in unknown ways with fluid such as blood

TABLE IV

Estimation of B (= g times Total Head) in Arteries (See text)

AORTA	$\rho \frac{V^2}{2}$	p	p	B	AREA OF CROSS-SEC.	$\pi a^2 B$
	ergs/cc	*mm Hg*	*dynes/cm²* ($\times 10^3$)	*ergs/cc* ($\times 10^3$)	*cm²*	*ergs/cm* ($\times 10^3$)
S 27	1500	55	73	74.5	0.242	18
S 29	560	68	90	91	0.502	26
	1200	58	77	78	0.502	39
S 37	321	46	61	62	0.408	25
S 35	660	42	56	57	0.282	16
Umbilical Artery S 25	824	76	101	102	0.160	16

which contains corpuscles and which flows in blood vessels. The development of turbulence in blood flowing in glass tubes has been a subject of recent study, however (Coulter and Pappenheimer, 1949). These authors used the radius, rather than the diameter as we have done, so their Re numbers are about one-half the value of ours.

Velocity pressure head of arterial blood flow. While this work was in progress, the absence of an important pressure gradient was not properly appreciated especially with respect to the determination of certain hydrodynamic factors. Consequently only a few observations were made in which velocity of flow and blood pressure were measured without one determination interfering with the other. Such instances are listed in Table IV.

The data of this table afford one an opportunity to calculate the "total head" at the place in the artery at which the measurements are taken. The total head is given by the expression

$$h = \frac{p}{\rho g} + \frac{V^2}{2g} + z,$$

where ρ is the density of the fluid, g is the acceleration of gravity, p is a mean pressure over the cross-section of artery, V is a mean velocity over the cross-section, and z is the elevation above some chosen level. In c.g.s. units, h will be given in cm.

If we take z to be the same for all places at which h is calculated, we may choose $z = 0$. Then

$$h = \frac{B}{\rho g},$$

where

$$B = p + \frac{\rho V^2}{2}.$$

B, whose dimensions are ergs per cc (or dynes per sq. cm.), would be constant at every point in an irrotational flow of a non-viscous incompressible fluid, V denoting, in that case, the velocity at a point.

The values of B for those observed flows in which the necessary data are available are shown in Table IV. It is seen that B varies from 57,000 to 91,000 ergs per cc, in the aorta. These figures are based upon values of mean velocity V read from the cineradiographs and values of p, taken as described above, which give the stress, at the wall of the vessel, normal to the wall.

The flows studied are, of course, not strict Poiseuille flows, since the latter presume a perfectly straight tube of circular section, and a steady flow of an ordinary viscous incompressible fluid (not loaded with suspended formed elements, as is blood). In the Poiseuille type of flow, the pressure is constant on a cross-section but decreases linearly in the direction of flow. Actually, a viscous fluid in motion does not, in general, have a pressure, in the sense that such a fluid at rest, or a non-viscous fluid in motion, does. Rather, the stress at a point requires for its description six numbers, instead of only one, and what is called pressure (and denoted here by p) is the arithmetic mean of the three principal stresses at the point. In a Poiseuille flow, the numerically distinct non-zero components are reduced to three and, it turns out, the stress normal to the wall is equal to p.

The velocity vector in a Poiseuille flow is everywhere parallel to the axis of the tube, and its single non-zero component is given by

$$u = \frac{P}{4\mu}(a^2 - r^2).$$

Here P is the drop in pressure per cm. of tube length, a is the radius of the tube, and μ is the viscosity of the fluid. The velocity is constant along any streamline, but its magnitude varies from a maximum on the axis to zero at the wall.

The mean velocity over a cross-section proves to be

$$V = \frac{P}{8\mu} a^2$$

In this case, we can readily calculate B for each point of the cross-section, and then find the average of B over the section. We get, for this average,

$$\bar{B} = p + \frac{2}{3} V^2$$

This differs a little from the formula for B calculated directly from the mean velocity, since the average of u^2 is not equal to V^2.

If we take the observed flows in the aorta and umbilical artery to be "nearly" Poiseuille in type, we find the differences between the value of B in Table IV and those calculated from the formula just given to be quite small, since in both cases the predominating term is p. The differences in B between aorta and umbilical artery are, essentially, merely the differences in pressure.

The energy density per cm., over a cross-section, is given by $\pi a^2 B$ (or, for all practical purposes, $\pi a^2 p$). Since energy is dissipated (as heat), we should expect $\pi a^2 B$ to be smaller in the more peripheral artery. (In Poiseuille flow,—the tube being neither curved nor branched and the flow steady,—the rate of dissipation of energy per second per cm of tube length is $\pi a^2 PV$.) The data available do not provide values for $\pi a^2 B$ in the aorta and umbilical artery of the same animal. However, the last column of Table IV shows that the differences, based upon values for different animals, are in the expected direction.

Pressure gradient in umbilical artery. The essential data are given in Table II which enable one to estimate the pressure gradient in the umbilical artery. These values are obtained by applying the Poiseuille equation and solving for P, the drop in pressure per unit length of blood vessel. This equation, which applies to flow of fluids in straight tubes only, is conventionally written as follows:

$$Q = \frac{\pi P a^4}{8\mu}$$

where Q is the volume flow in cubic centimeters per second; a, the radius of the tube; μ, the viscosity of the fluid, in this case assumed to be about 0.02. By rewriting this equation, P may be calculated as follows:

$$P = \frac{8\mu Q}{\pi a^4} = 0.051 \times \frac{Q}{a^4}$$

In Table V, this value is calculated for the five fetuses listed in Table II, in the same order. The rank according to diminishing radius is given in the second column of Table IV, and the approximate drop in pressure, P, is shown in the column at the right.

The pressure drop per centimeter length of umbilical artery is of the order of 0.5–1.0 millimeter per centimeter. This agrees well with values observed experimentally elsewhere (Reynolds, 1951). The lowest pressure drop is found in

the artery with the largest radius. Conversely, the artery exhibiting the greatest pressure drop is the one with the smallest radius. The other values, although not in entirely corresponding order, occupy intermediate positions with respect to the fall in pressure and volume flow of blood.

The balance of hemodynamic forces in the umbilical arteries may now be outlined in approximate terms. The calculated pressure drop is lower than the true value because it is calculated on the assumption that the artery is straight, and such usually is not the case. Curves or spirals in a vessel will occasion a greater pressure drop than in a straight vessel of equal length. Nevertheless the magnitude of the pressure drop would probably not be altered too greatly. We see from Table IV that some ninety-five per cent of the total head is made up of the pressure component, which we generally read as blood pressure. In addi-

TABLE V

Pressure drops in the umbilical arteries of fetuses listed in table II. Based on the Poiseuille equation (see text)

ANIMAL	RANK BY DECREASING SIZE OF RADIUS OF ARTERY	Q	RADIUS OF ARTERY	PRESSURE DROP PER UNIT LENGTH
		cc./sec.	*cm.*	*mm Hg/cm*
S 27	4	5.8	0.15	0.44
S 29	5	5.3	0.13	0.73
S 25	1	6.4	0.225	0.093
S 21	3	14.9	0.17	0.68
S 20	2	17.0	0.195	0.45

tion, for a one centimeter length of umbilical artery, the pressure drop may be of the order of 0.5–1.0 millimeter of mercury, corresponding to about 2 per cent of the total energy of the system. In round figures, therefore, we may say that about 90 per cent of the energy in the aorta is available for the purpose of propelling blood into the umbilical arteries. The system derives its impetus from the force of cardiac ventricular ejection, some of the energy being stored momentarily in the distended arterial walls near the heart and released as they recoil during diastole. This latter effect, as we have seen, is limited to the base and arch of the aorta.

DISCUSSION

The relation of visible pulsation to the pulse pressure wave

The observations reported above have shown that there is no detectable alteration in the calibre of large arteries, such as the umbilical artery and the descending aorta, with the pulse pressure. The "pulsation" which is observed on direct inspection is in fact caused by a bodily shift of the entire artery which is greatest at points of curvature, and the impression of movement is exag-

gerated since it is conveyed by alterations in the high lights reflected from moist surfaces. Moreover, when more than one point of curvature in the vessel is under observation the eye integrates the movements of the numerous high lights and the impression is obtained of a marked movement of the arterial wall. In cineangiograms of the abdominal aorta and renal arteries of sheep (unpublished data) it has never been possible to detect any lateral deformation of walls of these vessels. "Pulsation" has occasionally been visible but only in renal arteries which had a curved course, and in these cases the whole vessel was seen to be bodily displaced with each heart beat.

It would seem, therefore, that any change in calibre which may occur in arteries with the pulse pressure must be of a very small order. Kinmonth's (1952) work lends support to this view since he has shown that in main arteries, having a diameter of the order of 2.5 mm, a sustained rise of pressure of 30–50 mm Hg. is associated with an increase in diameter of 0.1 mm. Any alteration in calibre due to the smaller, faster and short pulse pressure would be smaller than this and dependent upon the frequency of the system, too.

Arterial diameter and a gradient of pressure. In order to estimate the pressure gradient, if existent, advantage was taken of the fact that the umbilical artery is distended under pressure when carrying blood and that it is always tending to collapse or constrict. This is known to all who handle the umbilical artery and are familiar with the extreme ease with which the vessels of the cord may be induced to collapse (cf. Barcroft, 1947). Two factors operate to favor collapse of the umbilical cord vessels.

One force is related to the morphological features of the Wharton's jelly of the cord; the other, to the contractile characteristics of the vessels themselves. When the blood vessels are normally filled with blood, the Wharton's jelly is drawn taut about them (Reynolds, 1952). This is less marked in the sheep cord than in the human, but there is some stretching of the connective tissue even in the sheep, so that this force tending toward collapse of the vessels contained within the cord is continually exerted when the blood vessels contain blood under pressure. Consideration of this fact suggested that if there is frictional loss of energy along the umbilical artery, then the pressure should be higher at the fetal end, near the heart, than at the distal or placental end of the cord. Moreover, the diameter of the artery at the fetal end would exceed that at the placental end. It was accordingly decided to photograph nearly the entire length of umbilical cord while it was carrying Thorotrast injected into the base of the aorta and to record pressure in the artery at the level of the first branch, as outlined above. In order to obtain information concerning the question of arterial deformation with pulsation, records of the pressure and the arterial diameters were obtained throughout about eight pulsations.

With respect to the point under consideration, that of the diameter of the

artery throughout its length, it was found that there is no measurable difference in diameter of the artery along its course (see Fig. 3). This is true at any phase of the pulse wave. It must be concluded on the basis of this evidence that, whatever the difference in pressure at the two ends of the artery, it is insufficient to affect the diameter at one end more than at the other.

What pictures taken at a much faster rate would show is, of course, not known. Although the rate of exposure in this part of the work was two frames per second, the actual duration of each exposure was 0.05 second. During the course of recording, some pictures were taken at the peak of systole (e.g., no. 7 in Fig. 3), at the end of diastole (e.g., no. 9 in Fig. 3), and at intermediate points of the ascending or descending limbs of the curve as shown by the indicated points in the graph of figure 4. Therefore, the fact that there was no measurable difference in diameter along the vessel, irrespective of the time in the pulse wave when the photograph was taken proves that the conclusion stated in the paragraph above is valid for situations in which events transpiring in a time range of 0.05 second or more are involved.

Venous pressure pulsation. The venous pulse is about one millimeter of mercury. This is superimposed upon an existing venous pressure of 20 to 40 or more millimeters of mercury when the blood is flowing freely and 6 to 10 millimeters when the column of venous blood is static. The venous pressure had been reported in earlier observations to vary by this much in different types of experiment (Barcroft, 1949; Reynolds, 1951). The present work shows that when blood is flowing freely, physiological venous pressure in the umbilical vein is of the higher rather than the lower value. Some of the physiological factors that may contribute to a high venous pressure in the umbilical artery have been discussed elsewhere and need not concern us here (Reynolds, 1952). What does arise from these observations as new is the demonstration of an inverse relationship between the arterial and venous pulses in the umbilical vessels.

A concept of the mechanism by which the mechanical force of arterial deformation is transmitted to the umbilical vein must rest upon two facts. First, the arteries and veins need not be contiguous, since they do not appose each other in the distended umbilical cord of the lamb and goat fetus. Second, the direction of the force applied to the vein is not one of compression resulting from arterial dilatation, but of elongation. This must mean that arteries elongate with pulse pressure, or they are displaced to one side or the other, since they do not materially enlarge in diameter. If they did increase in diameter the vein would be compressed and there would then be a positive correlation between the arterial and venous pressure curves instead of the inverse one observed in this work.

It would appear that as the umbilical arteries are elongated by summation of multiple, minute local elongations, especially where the vessel spirals or

bends, the vein is passively and momentarily stretched. This could account for the small and transitory decrease in venous pulse pressure as arterial pressure increases. It is not improbable that there are other more basic hydrostatic factors in the pattern of arterial distortion with pulse pressure (e.g. increase in total length of artery by longitudinal stretching) which have not been revealed in these studies and which contribute to the effect observed in venous pressures. The consequence on the venous pulse would be similar to that already described, but the operation of such additional factors is hypothetical so further reference to them may be omitted until the matter is investigated.

Stream-line pulsation. In the study of stream-line blood flow in this work, a rhythmic pulsating component has been described. It was seen in arterial flow at those points where the direction of the stream-line was reflected by a curving surface of the intima along the long axis of the artery. In order to demonstrate this, the quality of the picture must be clear, as in direct cineradiography, and the speed of recording must be such that the pulsation is not blurred. The existence of rhythmic pulsation in straight or forward stream-lined flow has not been observed in this work. It must exist, but the technics employed were insufficient to permit its demonstration.

It is probable that the speed of recording successive frames of pictures in this work is too slow to allow visualization of the pulsating character of forward flow in straight arteries. The requirements for such a demonstration may be judged from the following considerations. A pressure pulse wave in the sheep fetus is about one-third of a second in duration. The ascending limb of the wave is about one-third of this, or about 0.1 second. If ten pictures of the accelerating (i.e., increasing pressure) phase were to be taken, the frequency of recording would have to be about one hundred frames a second. For five frames per accelerating phase of the wave the frequency would have to be about fifty frames a second. The latter would doubtless be too slow for accurate work. Only by technical improvement in the speed of photography, while retaining the clarity of the pictures as in this study will a really adequate analysis of pulsatile flow in arteries be achieved.

Umbilical vein blood flow. In view of the results described in this paper, what, if anything may be said concerning the fundamental nature of the flow of blood from the fetus to the placenta and then back to the fetus respectively? It is clear that loss of energy from friction is negligible since the diameter of the arteries and veins is large enough to preclude appreciable loss of energy from such a factor. It would seem, accordingly, that one of the primary functions of pressure within the umbilical vein is to keep its diameter large. Thus the fact that the cord may be both long and tortuous will not affect adversely venous return of blood from the placenta.

So long, therefore, as the supply of blood in the placenta is infinitely large

and the capacity of the fetus is also infinitely large (as in effect is the case because of the adequacy of the placental-fetal circulation) it appears that a difference in pressure of even a very slight amount between the two ends of the umbilical veins will allow the blood to pour from fetus to placenta and from placenta to fetus.

In the umbilical artery, there is as we have seen appreciable energy imparted by the momentum of the rapidly moving blood stream. This velocity-head must assure that there will be rapid and voluminous flow from the fetus to the placenta without too much frictional loss along the way. In the vein, the velocity of flow is somewhat less because the cross-sectional diameter of the vein is greater (Reynolds, 1952). But in the vein the flow is assured because the blood is fed into the vein rapidly and continually from the placental capillaries, and it drains away as rapidly into the fetal circulatory system where the lower venous pressure assures directional flow toward the fetal heart. Superimposed upon the pressure in the umbilical vein is the small, rapid, pulsating pressure arising from arterial deformation. This cannot help but exert a mechanical pumping action in the manner of a pistonless pump. The extent to which this adds force to the other fundamental mechanism to account for blood flow between the placenta and fetus described above cannot be estimated at the present time although it would appear to play a minor role.

Volume flow of blood to placenta and fetal age. It will be seen in Table II that the linear velocity of flow varies but not strictly inversely with the diameter of the artery. The minute volume of flow is very variable. It is greatest in the two largest and full-term lambs. Such variation is in agreement with observations of Cooper, Greenfield and Huggett (1949), who used a venous occlusion method to study umbilical blood flow. However, our results differ from theirs in one respect. Our flow-rate per kilogram of fetal weight is definitely higher than theirs. They report one high value of about 290 cubic centimeters per kilogram of fetal weight. Three of our values are higher than this. Moreover, the rates of flow determined by us did not show a tendency, as did theirs, to decline with increasing fetal age (or weight). On the contrary, the unit rate of flow per kilogram was greatest in our largest fetuses. The present data are few in number, but there is quite good agreement among them. Moreover, the experiments were carried out without obstruction to, or manipulation of, the cord. It is possible that the lower values in fetuses near term reported by Cooper, Greenfield and Huggett reflect the greater tendency of the cord blood vessels near term to contract upon manipulation (Barcroft, 1947). It is possible, too, that fetal heart rate and output decrease in response to the venous occlusion method of flow rate determination in the cord. This is an established fetal heart response to cord occlusion (Reynolds, 1951).

The proportion of blood passing down the aorta which enters the umbilical arteries has never before been estimated. What may be called reasoned specu-

lation by Barcroft and his associates (1947) led them to conclude that about two-thirds of the cardiac output passes to the placenta. In the present work, the same technic that was used for estimating the volume flow of blood traversing the umbilical arteries was applied to determination of the rate and volume of blood flow down the aorta. The approximate figures, given in Table II, show that the flow in the aorta is about 50 per cent greater than that which goes to the placenta. That is to say, about two-thirds of the aortic flow enters the umbilical arteries and the rest supplies the abdominal viscera and the caudal somatic parts of the body. The present data suggest, therefore, that rather less than two-thirds of the cardiac output of the fetus goes to the placenta if additional allowance is made for blood going to the anterior end of the fetus as well.

Mention may be made in connection with the present data of another apparent relationship. The amount of blood flowing to the placenta per kilogram of fetal bodyweight is approximately the same in the different experiments, despite the presence of umbilical arteries of very different calibres. This variability in size affects in turn the velocity of blood flow in them. It therefore appears that, so long as the diameter of the arteries is not so small that it seriously reduces through friction the rate of flow, the amount and rate of blood flowing to the placenta is not determined by arterial size but by other factors related to the requirements of the fetus, such as the fetal cardiac output.

SUMMARY

1. Observations of blood pressures and stream-lining in umbilical cords of the fetal lamb and of deformation of umbilical arteries and veins have been recorded.

2. Insertion into an umbilical artery of a needle for recording blood pressure unavoidably distorts the wall of the vessel and stream-lines of flow. For this reason true pressures like those in the intact artery cannot be recorded. True lateral pressures were measured from the first branch of an umbilical artery. The vessel was fixed at right angles to the axis of the main vessel and the pressure transmitted by means of a capacitance manometer for recording.

3. When a long segment of umbilical cord was directly photographed by x-ray moving pictures, using Thorotrast to visualize the vessel, and while recording pressures as described above, it was found that at no time in the pulse pressure wave was there a demonstrable change in diameter of the artery. Similarly, there was no difference in the diameter of the artery along its course as would be expected if, due to frictional loss of energy, there were a significant decrease of pressure within the artery.

4. Pressure pulses were recorded in the umbilical artery and umbilical vein simultaneously. They were 180° out of phase with each other since the pulse pressure in the vein decreased as that in the artery increased, and vice versa.

5. Study of stream-lines of blood flow by x-ray cineangiography show that as a quick velocity component of the moving fluid during an arterial pulse wave hits part of the umbilical artery, the vessel may be displaced slightly. Such movement gives rise to visible arterial pulsation. There is no increase in arterial diameter measurable by the methods as used in this study (except in the arch of the aorta) i.e., in direct cineangiographs recorded at a frequency of twenty-frames per second.

6. Normal umbilical vein pressure, with unobstructed flow is high, being equivalent to 30–40 millimeters of mercury.

7. The velocity and volume flow of blood through the umbilical arteries is recorded. The velocity is very variable, being inversely related to the diameter observed. The volume of flow in the two arteries per kilogram of body weight per minute is between 300 and 400 cubic centimeters. This is about two-thirds the volume that goes through the descending aorta.

8. The basis of the venous pulse in the cord and of the stream-line blood flow in the umbilical cord, and the nature of venous return from the placenta to the fetus are discussed briefly.

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THE HEPATIC SYNTHESIS OF CHOLESTEROL IN THE PREGNANT RAT*

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Studies (1) from this laboratory have indicated that the liver furnishes all or the major portion of the cholesterol present in the blood of the rat. This finding recently has been confirmed in the dog by Gould *et al.* (2). It also was observed in this laboratory (3, 4, 5) that the biliary excretion of cholesterol furnishes an index of the "rate" of hepatic synthesis of cholesterol. Thus, a simple means was made available whereby the rate of hepatic synthesis of cholesterol could be measured in various physiological and pathological states. The present report describes the rate of hepatic synthesis of cholesterol in the rat before, during, and immediately after pregnancy.

METHODS

Female rats (Long-Evans strain), aged eight to twelve weeks, were permitted to become pregnant. Approximately midway through gestation, 18 pregnant rats were subjected to bile duct cannulation and a similar procedure was done in 10 rats, 24 to 48 hours postpartum. In addition, there were 17 rats used as controls. Bile was collected during a 24 hour period in which food was withheld, and analysis was made of the cholesterol content by methods described previously (6). Samples of plasma were obtained prior to pregnancy and again prior to bile duct cannulation and were analyzed for their concentration of cholesterol (7).

RESULTS

The Cholesterol Changes in Rats Before, During, and After Gestation

1) *In Plasma.* No significant change apparently occurred in the plasma cholesterol of the 18 pregnant rats. Thus (see Table I), although their average plasma cholesterol was 57.0 mg. per 100 ml. (S.E. Mean: ±3.2 mg.) and their pre-pregnant average value was 48.7 mg. per 100 ml. (S.E. Mean: ±2.9 mg.), calculation of the S.E. of the Difference of the Means (±4.3 mg.) suggests that the observed difference in the values may not be significant. Moreover, comparison of the mean plasma content of the 17 control rats (57.3 mg./100 ml.) with that of the pregnant rats indicates no difference. On the other hand, the average plasma cholesterol of the 10 rats, 24 hours after gestation (65.04 mg./100 ml.), when compared with that obtained before pregnancy (53.3

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mg./100 ml.) or with that observed in the control rats (57.3 mg./100 ml.) shows a slight rise which appears to be statistically valid.

2) *In Bile.* As Table I indicates, the difference in the daily volume of bile in the normal control and pregnant rat does not appear to be statistically significant [3 × Standard Error of Difference of Means (2.7) is greater than the difference in Means (1.2)]. There does appear to be a significant increase, however, in the volume of bile excreted by the post-gestational rat.

The cholesterol concentration of bile (8.4 mg./100 ml.) in the 18 pregnant rats was reduced markedly when compared with that found (13.9 mg./100 ml.) in the 17 control rats. This reduction in concentration led to the decreased

TABLE I

Effect of Pregnancy on Bile Cholesterol Excretion

TYPE OF RAT	NO. OF RATS	WEIGHT	PRE-PREGNANT PLASMA CHOLESTEROL CONCENTRATION	PLASMA CHOLESTEROL CONCENTRATION*	BILE		
					Daily Volume	Cholesterol	
						Concentration	Content
		(gm.)	(mg./100 ml.)	(mg./100 ml.)	(ml.)	(mg./100 ml.)	(mg./24 hrs.)
Control	17	202	—	57.3	14.1	13.9	1.85
Range:		(165–240)		(37.3–77.0)	(10.0–25.0)	(9.0–18.5)	(1.26–2.98)
S.E. Mean:				±3.2	±0.78	±0.68	±0.12
Pregnant	18	215	48.7	57.0	12.9	8.4	1.1
Range:		(171–240)	(37.0–57.0)	(36.0–72.0)	(8.5–17.5)	(4.1–13.5)	(0.5–2.6)
S.E. Mean:			±2.9	±3.2	±0.60	±0.52	±0.10
24 hours post-partum	10	214	53.3	65.04	17.1	11.6	1.97
Range:		(194–245)	(33.0–83.0)	(50.0–83.4)	(14.0–19.6)	(9.0–15.9)	(1.6–2.8)
S.E. Mean:			±4.0	±0.8	±0.54	±0.72	±0.13

* At time of bile duct cannulation.

excretion (1.1 mg./24 hours) observed in these rats. This finding of a reduced biliary concentration and excretion of cholesterol, despite the normalcy of the plasma cholesterol, is quite understandable when it is remembered that biliary cholesterol has been found (3, 4) to be *completely independent* of plasma cholesterol concentration.

DISCUSSION

The results of the present study indicate that, although little or no change occurred in the plasma cholesterol of the rat during pregnancy, a marked decrease in biliary concentration and excretion of cholesterol occurred. In view of the data (3, 4, 5) suggesting that this latter mechanism varies as does the rate of hepatic synthesis of cholesterol, it would appear that there is a depression in the manufacture of cholesterol by the liver of the pregnant rat.

Elsewhere evidence was presented (8) showing that administration of estrogens to rats similarly depressed biliary cholesterol concentration and excretion, also suggesting that estrogen administration significantly depressed the rate of hepatic synthesis of cholesterol. Since there is an increase of estrogen levels during pregnancy (9), the depressed rate of hepatic cholesterol synthesis found in the pregnant state might be related to increased estrogen levels. These findings are of interest in view of evidence that cholesterol metabolism may be altered by estrogen therapy and by the advent of pregnancy (10–13).

SUMMARY

Using the biliary cholesterol concentration as an index of hepatic synthesis of cholesterol, the latter has been studied in pregnant and postpartum rats. The results obtained suggested that a significant depression of the rate of hepatic synthesis of cholesterol occurred in the pregnant rat, with a return toward normal early in the postpartum state. The possible mechanism of these changes was discussed, with particular reference being made to the possibility that the high estrogen levels occurring in the pregnant state might be responsible.

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THE OCCURRENCE OF FOCAL TUBERCULOID LESIONS IN EXPERIMENTAL SERUM SICKNESS

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It has long been known that granulomatous tuberculoid lesions (i.e., lesions consisting of focal accumulations of coherent epithelioid cells with or without multinuclear giant cells), usually in or adjacent to the walls of affected arteries, are encountered in some cases of periarteritis nodosa in man (1). More recently it has been established, both by experimental and by clinical-pathological observations, that periarteritis nodosa can result from anaphylactic hypersensitivity (2), and, following this, striking tuberculoid lesions have been observed in some of the human cases of periarteritis nodosa that have clearly been caused by anaphylactic hypersensitivity to various drugs. The writer (3) has reported the occurrence of lesions of this type in a case of periarteritis nodosa resulting from hypersensitivity to iodine; Van Wyk and Hoffmann (4) in a case of periarteritis nodosa caused by hypersensitivity to dilantin sodium; Fingerland (5) in a case of periarteritis nodosa now clearly recognizable as having resulted from hypersensitivity to neosalvarsan. In addition, we have observed lesions of this type in some of the cases of periarteritis nodosa caused by hypersensitivity to sulfonamides that have come to autopsy in this department. It is therefore clear that in man tuberculoid lesions can occur in association with the necrotizing-inflammatory arterial lesions produced by anaphylactic hypersensitivity.

In the experimental animal, granulomatous accumulations of mononuclear cells and multinuclear giant cells develop during the serum sickness type of protracted anaphylactic reaction, particularly in the endocardium (6), but these have hardly exhibited a frank tuberculoid character. In the experiments of Rich and Gregory (7) carried out some years ago on the rôle of anaphylactic hypersensitivity in the pathogenesis of periarteritis nodosa, tuberculoid reactions were occasionally encountered in the spleens of rabbits subjected to serum sickness, but these were not sufficiently well-marked or frequent to justify either mention or a conclusion as to their relation to the sensitizing procedure. In subsequent experiments in which a study has been made of the tissues of a large number of rabbits subjected to serum sickness by the same method, definite tuberculoid, granulomatous lesions have been encountered with sufficient frequency to establish their relationship to the sensitizing procedure. We have never encountered lesions of this type in unsensitized control rabbits. We have briefly reported the experimental production of

these tuberculoid lesions in a discussion of the types of lesions that can be caused by anaphylactic hypersensitivity (10), and also in a discussion of the pathogenesis of the tubercle (12). The purpose of the present paper is to define and illustrate their nature in somewhat greater detail.

The animals used in these studies have been male, albino rabbits. Protracted anaphylactic reactions of the serum sickness type were produced by a single intravenous injection of sterile horse serum, without preservative,[1] in a dosage of 10 cc. per kilogram of body weight. This procedure permits some of the antigen to remain in the circulation until antibody makes its appearance, and favors the development of a protracted anaphylactic reaction of the serum sickness type (7). The animals were killed, by etherization, from the twelfth to the sixteenth day following the injection of the serum.

In these experimental animals, while loose collections and occasional coherent aggregates of epithelioid cells may make their appearance in lymph nodes (Fig. 11), pronounced tuberculoid lesions have so far been found only in the spleen. Some of the lesions are sharply perivascular, appearing as a tissue of epithelioid cells, with occasional multinuclear giant cells, completely or partially encircling the arteries of some of the splenic Malpighian bodies (Fig. 1). Tuberculoid lesions of the same character occur in the same location in some cases of periarteritis nodosa resulting from drug hypersensitivity in man (Fig. 2). These perivascular tuberculoid lesions are often adjacent to foci of necrosis in the arterial wall, but they also often surround a normal-appearing cross section of an artery. It is, of course, possible that the wall of such an artery may be damaged at a point somewhat further along in its course, and that the perivascular tuberculoid reaction is continuous with that incited at the immediate site of vascular damage. However, the tuberculoid reaction is not confined to the region immediately adjacent to the arteries. Often, sheets of epithelioid cells with associated multinuclear giant cells develop at the outer margins of Malpighian bodies, sometimes forming rings which encircle the inner lymphoid tissue of the Malpighian bodies (Fig. 3). In some cases the tuberculoid tissue replaces most of the normal structure of the Malpighian body and extends into the adjacent pulp tissue (Figs. 4, 5, 6, 7). Occasionally, small foci simulating caseation occur in both the experimental and the human tuberculoid lesions (Figs. 9, 10).

Although, in the animals subjected to serum sickness, well-marked tuberculoid lesions have so far been encountered only in the spleen and in an occasional lymph node (Fig. 11), in the human being with periarteritis nodosa resulting from hypersensitivity to drugs perivascular tuberculoid lesions may occur in other tissues as well. In the spleen in such cases, a tuberculoid reaction may occur not only about the arterioles (Fig. 2) and in the Malpighian

[1] The horse serum was very kindly provided by Sharp and Dohme, Inc.

bodies, but may also be prominent along the margins of trabeculae that have undergone collagen degeneration in the manner described by Rich (3) and by More, McMillan and Duff (13) as a result of drug hypersensitivity. In patients with drug hypersensitivity, striking tuberculoid lesions may also occur in lymph nodes, particularly in association with the focal necrosis of lymphoid tissue that we have described as an effect of protracted reactions of the anaphylactic type in man (2).

Focal granulomatous tuberculoid lesions in various sites have been described in human cases of severe asthma with periarteritis nodosa (see, for example, Fig. 1, in ref. 8), and we have recently studied at autopsy (No. 23061) a case of asthma in which there were widespread focal tuberculoid lesions composed of epithelioid and giant cells (Fig. 12), in addition to periarteritis nodosa, extensive eosinophilic pneumonitis and myocarditis, and multiple foci of collagen degeneration bordered by palisading mononuclear cells, the latter lesions reproducing in miniature the appearance of subcutaneous rheumatic or rheumatoid nodules. Similar cases have been described by Bergstrand (8), Churg and Strauss (9), Pagel (11) and others.

While it is clear, from the experimental lesions and from human material, that anaphylactic hypersensitivity can give rise to focal tuberculoid lesions, the exact pathogenesis of the lesions is not yet clear. In man, the tuberculoid reaction in cases of anaphylactic hypersensitivity often appears to be incited by altered collagen, as in the case of the tuberculoid lesions bordering the degenerated splenic trabeculae mentioned above, or adjacent to the degenerated segment of the wall of a vessel affected by periarteritis nodosa. It is well known that a tuberculoid reaction may surround pieces of deteriorated collagen in rheumatic and rheumatoid subcutaneous nodules (Fig. 13). However, most foci of deteriorated collagen in hypersensitive reactions do not provoke a tuberculoid reaction. Why a tuberculoid reaction occurs at some sites of deteriorated collagen and not at others that appear identically altered, morphologically, in the same body, remains obscure; and until we learn more about the local chemical and physical alterations at the different sites of collagen damage, and more about the conditions that promote the transformation of macrophages into epitheloid cells and giant cells, speculation regarding this matter is of little value. Furthermore, in both the human being and the experimental animal, tuberculoid reactions associated with anaphylactic hypersensitivity occur at various sites at which there is no degree of primary collagen degeneration sufficient to provide a persuasive reason for regarding the development of the tuberculoid tissue at those particular sites as having resulted from the presence of degenerated collagen. This is strikingly true of the tuberculoid lesions that occur in the experimental animal at the outer margins of the splenic Malpighian bodies.

Why tuberculoid lesions occur in some cases of protracted anaphylactic reactions, and not in others produced by the same antigen, is likewise obscure. While it may be that one antigen is more effective than another in inducing a hypersensitive tuberculoid reaction, there can be no doubt that different individuals differ in their tendency to react in this manner to a given antigen; for among a series of animals given the same dosage of the same antigen per kilogram of body weight and killed two weeks later, marked lesions of this type will be present in the random sections of some of the animals, only moderate lesions in others, and in some no true tuberculoid tissue at all, though rare single epithelioid cells or groups of two or three epithelioid cells may be present. The same is true of human beings who develop protracted anaphylactic reactions of the serum sickness type to a given sensitizing drug. Thus, only some of those who develop periarteritis nodosa as a result of sensitization to a particular drug will develop concomitant tuberculoid foci.

TABLE I

Comparison of the Incidence and Degree of Tuberculoid Lesions, Periarteritis Nodosa and Glomerulonephritis in 60 Rabbits Killed Two Weeks after an Intravenous Injection of 10 c.c. of Sterile Horse Serum per Kilogram of Body Weight

DEGREE OF LESIONS*	TUBERCULOID LESIONS	PERIARTERITIS NODOSA	GLOMERULONEPHRITIS
+++	8 (13%)	12 (20%)	12 (20%)
++	7 (11.6%)	17 (28%)	9 (15%)
+	14 (23%)	14 (23%)	13 (21.6%)
0	31 (51.6%)	17 (28%)	26 (43%)

* Cases with only rare, single epithelioid cells, or occasional groups of only two or three epithelioid cells are included in the negative group.

Furthermore, neither in the human being nor in the experimental animal is there any significant correlation between the development or degree of anaphylactic tuberculoid lesions and of cardiovascular or renal lesions of hypersensitivity (Tables I and II). This is another of the many instances in which an un-understood individual "disposition" governs the type of lesion that the hypersensitive body will develop in response to a given antigen. It can be seen that, in the particular series of animals listed in Table II, while all animals that developed in the most marked tuberculoid lesions (Group A) had periarteritis nodosa, the degree of the latter lesions varied in these animals from marked to slight. On the other hand, among the animals with the most marked lesions of periarteritis nodosa (Group B), and among those with the most marked lesions of glomerulonephritis (Group C), the degree of tuberculoid lesions ranged from marked to zero.

It may be stated that the development of tuberculoid foci in the experimental animals bore no relation to the antibody titre (antigen-dilution pre-

cipitin test) measured two weeks after the sensitizing injection, or to the degree of sensitization as measured by the reaction of the skin to the intradermal injection of 0.1 cc. of the sensitizing antigen. Indeed, in a series of 20 animals in

TABLE II

Comparison Between Degree of Tuberculoid Lesions, Periarteritis Nodosa and Glomerulonephritis in Individual Animals of Table I

GROUP*	RABBIT	TUBERCULOID LESIONS	PERIARTERITIS NODOSA	GLOMERULONEPHRITIS
A	62	+++	+++	+++
	70	+++	+	++
	80	+++	++	++
	103	+++	++	++
	113	+++	+	0
	115	+++	++	0
	117	+++	+++	0
	118	+++	++	+++
B	21	0	+++	+++
	30	+	+++	+++
	33	+	+++	+++
	36	+	+++	+++
	61	+	+++	+++
	62	+++	+++	+++
	64	+	+++	0
	66	+	+++	0
	76	+	+++	0
	104	++	+++	+++
	107	+	+++	+
	117	+++	+++	0
C	21	0	+++	+++
	25	0	++	+++
	30	+	+++	+++
	33	+	+++	+++
	36	+	+++	+++
	40	0	++	+++
	61	+	+++	+++
	62	+++	+++	+++
	104	++	+++	+++
	118	+++	++	+++

* Group A—All animals of Table I having marked tuberculoid lesions. Group B—All animals of Table I having marked periarteritis nodosa. Group C—All animals of Table I having marked glomerulonephritis.

which the sensitizing stimulus was limited to a single intracorneal injection of about $\frac{1}{10}$ cc. of horse serum, the reaction to the skin test two weeks later was strong in all animals and extremely marked in a fourth of them, the antibody titres were, on the average, high, but no tuberculoid foci were found in the spleens of any of these animals killed at that time. So far, we have observed

the splenic tuberculoid reaction only in animals in which the sensitizing antigen has been administered intravenously, and under conditions which permit some of the antigen to be present in the blood stream after sensitizing antibody has made its appearance.

Finally, it is of interest to mention that the spleens of 57 rabbits that were treated with ACTH or cortisone in experiments dealing with the effect of these hormones upon the cardiovascular and renal lesions of hypersensitivity (14) (15) (16), were examined for the presence of tuberculoid foci. All of these animals received an intravenous sensitizing dose of 10 cc. of sterile horse serum per kilogram of body weight, and were treated from the beginning to the end of the experiment with 10 mgm. of ACTH per day in divided dosage, or with a daily injection of 7.5 mgm. of cortisone. The details of these experiments may be found in the papers referred to. For the present purpose it suffices to state that there was no significant difference in the number of ACTH-treated animals that developed tuberculoid lesions as compared with their sensitized, untreated controls, but the lesions were, on the average, rather less marked in the treated animals. The cortisone-treated animals, however, showed a significantly lower incidence of occurrence and degree of development of tuberculoid lesions than did their controls. It may be stated that in those experiments, with the dosages used, cortisone produced more pronounced metabolic effects (lipemia, deposition of glycogen and fat in the liver) than did ACTH.

SUMMARY

Tuberculoid lesions, consisting of aggregates of epitheloid cells with or without multinuclear giant cells, occur in the spleen in rabbits sensitized anaphylactically under conditions which permit antigen to be present in the circulation after antibody has made its appearance. There is an individual difference in the tendency of different animals to develop this tuberculoid reaction under the same conditions of dosage and method of administration of the same sensitizing antigen; and under the conditions of these experiments there was no close correlation between the development of the tuberculoid lesions and the antibody titre, the degree of hypersensitivity as measured by the skin test, or the degree of cardiovascular or renal lesions of hypersensitivity. The occurrence of tuberculoid lesions in instances of human anaphylactic-type hypersensitivity is discussed.

The development of tuberculoid lesions during sensitization to soluble, non-lipid antigens is of considerable interest in relation to the as yet unsolved problem of the pathogenesis of tuberculoid lesions in the infections in which they occur.

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EXPLANATION OF ILLUSTRATIONS

(Photographs by Miss Joan Wheaton)

FIG. 1. Epithelioid cells (giant cell at top) surrounding artery of splenic Malpighian body of serum sickness rabbit with periarteritis nodosa in other tissues (No. 117).

FIG. 2. Epithelioid cells partially surrounding artery of splenic Malpighian body in human case of periarteritis nodosa caused by hypersensitivity to dilantin sodium (tissue from case described in ref. 4).

FIG. 3. Arc of tuberculoid tissue at outer margin of splenic Malpighian body in serum sickness rabbit (No. 113).

FIG. 4. Tuberculoid tissue in splenic Malpighian body and extending into pulp in serum sickness rabbit (No. 113).

FIG. 5. Nodules of tuberculoid tissue in spleen of serum sickness rabbit (No. 117).

FIG. 6. Higher magnification of tuberculoid tissue of Fig. 5.

FIG. 7. Broad zone of tuberculoid tissue surrounding lymphocytes of splenic Malpighian body of serum sickness rabbit (No. 103). At this low magnification the individual cells are not distinctly visible, but the entire broad zone surrounding the central black lymphocytes consists of characteristic tuberculoid tissue. Tubercle-like nodule at left.

FIG. 8. Giant cells (top) and epithelioid cells in spleen of serum sickness rabbit (No. 104).

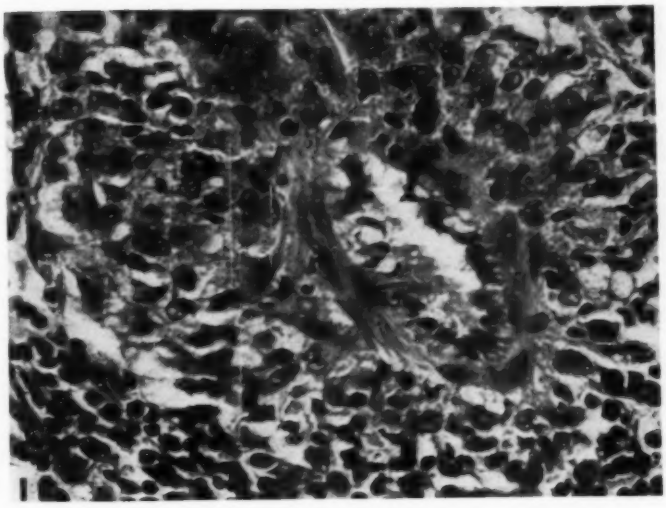
FIG. 9. Small focus simulating caseation in perivascular tuberculoid tissue in spleen of serum sickness rabbit (No. 33).

FIG. 10. Tubercle-like nodule with epithelioid cells, giant cells (arrows) and central necrosis simulating caseation, in spleen of human case of periarteritis nodosa caused by sulfonamide hypersensitivity (Autopsy #17805). Identical tuberculoid foci were present in the spleen in the case of iodine hypersensitivity described in reference 3.

FIG. 11. Tuberculoid focus in lymph node of serum sickness rabbit (No. 33).

FIG. 12. Tuberculoid nodule of epithelioid and giant cells in the capsule of a lymph node of the human case of asthma, periarteritis nodosa, eosinophilic pneumonitis and myocarditis mentioned in text (Autopsy 23061).

FIG. 13. Tuberculoid tissue surrounding swollen and degenerated collagen fibers in a subcutaneous nodule from a case of rheumatoid arthritis.



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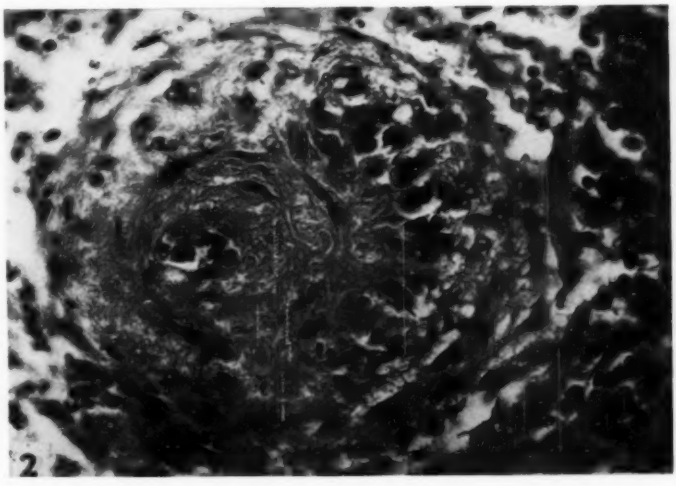

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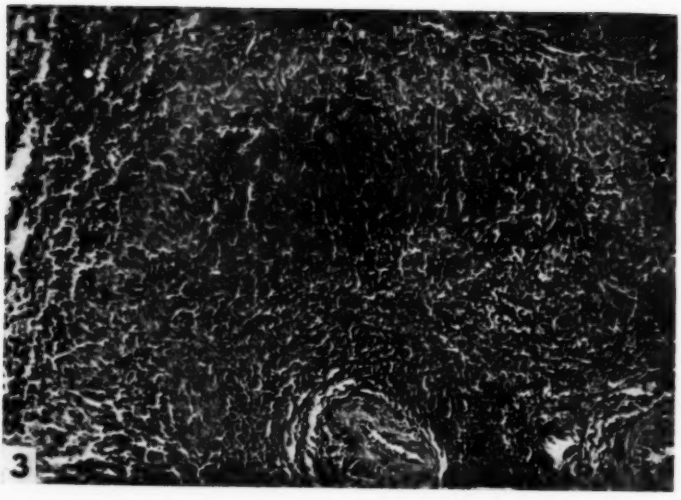

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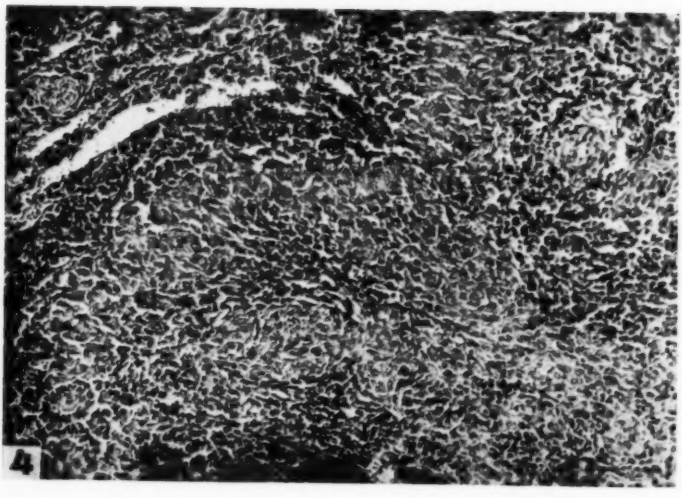

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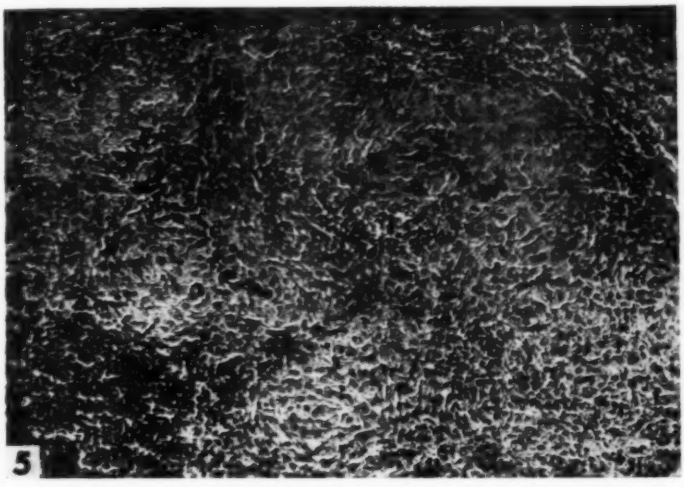

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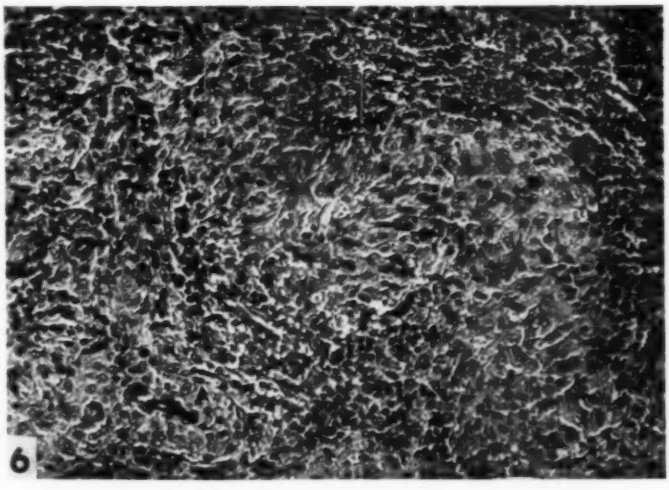

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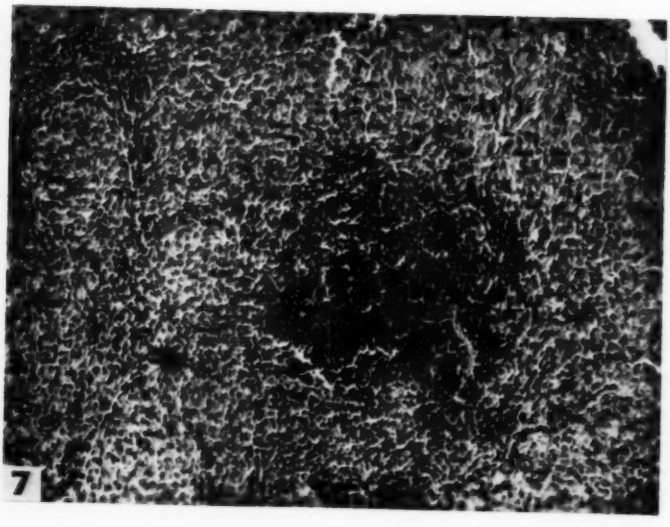

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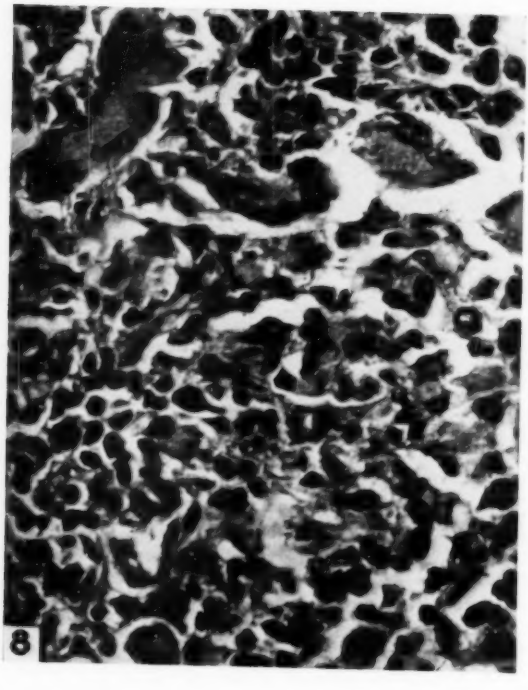

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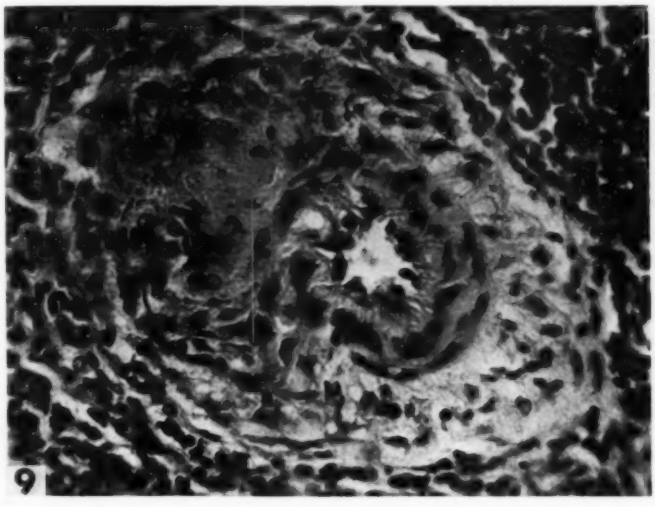
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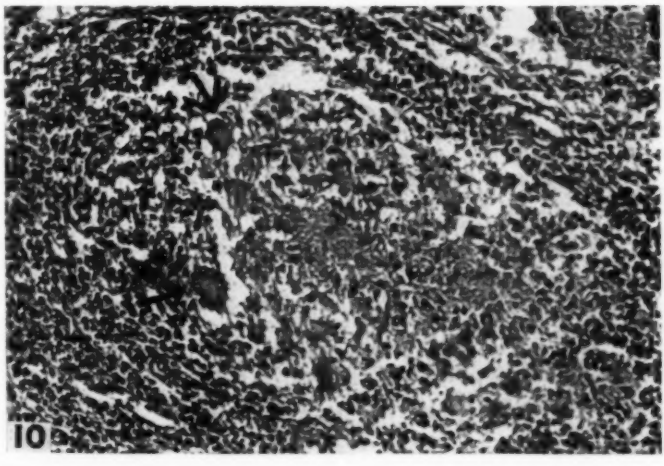
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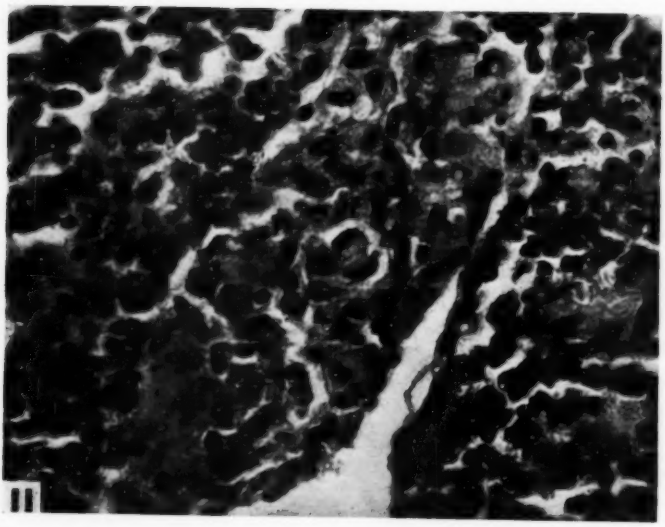
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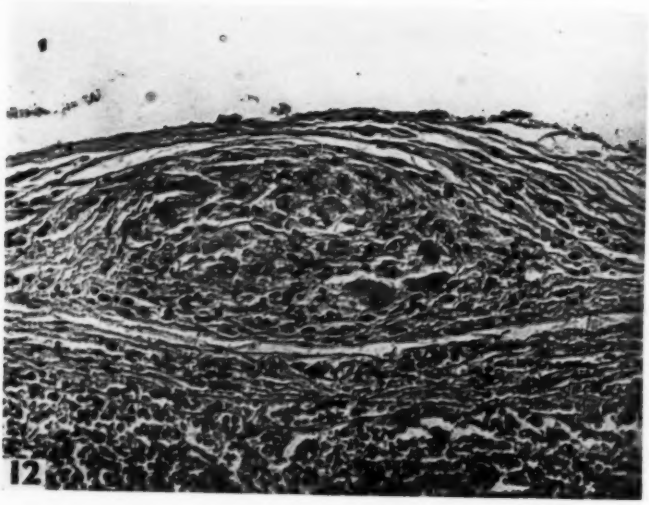
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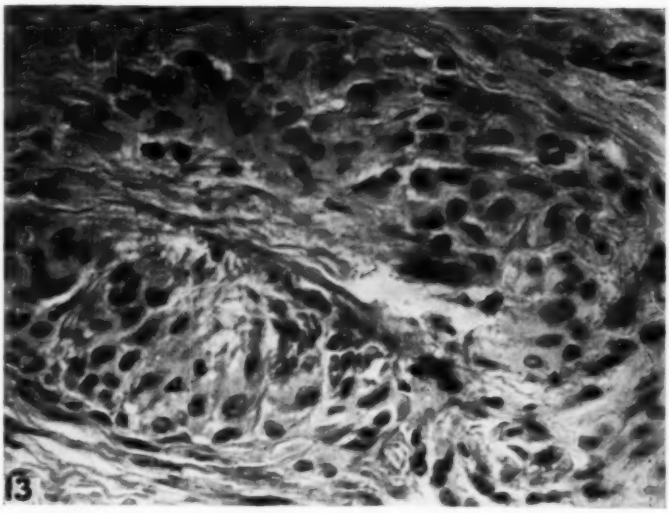
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EFFECT OF ADRENOCORTICOTROPIC HORMONE (ACTH) AND CORTISONE ADMINISTRATION IN PATIENTS WITH MYASTHENIA GRAVIS

AND

REPORT OF ONSET OF MYASTHENIA GRAVIS DURING PROLONGED CORTISONE ADMINISTRATION[1]

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The administration of adrenocorticotropic hormone (ACTH) to patients with myasthenia gravis has been reported to produce a decrease in strength in most patients during the period of hormone administration (1–6). A significant increase in strength and decrease in neostigmine requirement beginning within a week after cessation of ACTH has been described in one, ten of fifteen, one of two, and ten of ten patients (7, 2, 3, 6), and after cortisone in one of three patients (3). The improvement is said to have been more notable in moderately or severely ill patients than in those with mild weakness, and to have persisted for weeks or months (2). Other observers have noted no improvement in four subjects following ACTH or cortisone (4, 5, 8). In the observations to be described the effect of the administration of ACTH to ten myasthenic patients, and of cortisone to three, was studied. A decrease in strength and in response to neostigmine was observed in a majority of these patients during, and, in most instances, following hormone administration. In none was an increase in strength observed either during or following hormone administration.

Weakness of mild or moderate degree has been observed during the administration of ACTH or cortisone to non-myasthenic subjects (9, 10), but the onset of myasthenia gravis during the administration of these hormones has not been reported. One instance of this association will be described.

PROCEDURE

The myasthenic patients who were studied ranged in age from 12 to 56. In seven of the twelve patients the duration of illness was less than a year. In only one patient (L. K.) was there a history of partial spontaneous remission, which had followed thymectomy 9 years before. One other patient (A.M.) had been subjected to thymectomy, four years previously, without any effect on his illness. The degree of bulbar and peripheral weakness,

[1] This work was supported by a contract between the Chemical Corps Medical Laboratories, Army Chemical Center, U. S. Army, and the Johns Hopkins University. Part of the cortisone and ACTH employed was purchased through a grant-in-aid from the Division of Research Grants and Fellowships, National Institutes of Health, U. S. Public Health Service.

and the neostigmine or tetraethyl pyrophosphate (TEPP, (11)) requirement, are recorded in Table I. Three patients responded poorly to large doses of neostigmine. One had received no medication prior to ACTH administration.

Observations on muscle strength and endurance were made each morning when the patient was in the basal state, five or more hours after the last dose of neostigmine or TEPP, and during the day, following administration of these drugs. In two patients who required intramuscular neostigmine at two hour intervals observation of true basal strength was not feasible. Likewise, in the three who received TEPP this could not be determined, because of the prolonged action of the drug. In these five patients the maximum degree of weakness has been recorded as the basal strength. Daily observations were made for one to three weeks prior to ACTH or cortisone, during the administration of these hormones, and for 2 to 12 weeks (average 5 weeks) after administration. The patients were then examined at intervals over a period of 2 to 34 months (average 16 months).

Seven patients were given 80 to 100 mg. of ACTH intramuscularly daily for four to six days. This dosage range was employed by the investigators who have reported the highest incidence of remission following ACTH (1, 2). One patient received 100 mg. a day for three days, one 40 mg. a day for four days, and one an average of 112 mg. a day for eleven days. One patient was given two courses of ACTH. Two patients were given an average of 150 and 200 mg. of cortisone intramuscularly daily for eleven and twelve days. One of the patients who had received ACTH was later given an average of 200 mg. of cortisone daily for eight days. Whenever the period of hormone administration exceeded four days the daily dose was progressively reduced on the last three or four days. The doses of hormone that were administered produced a reduction in the number of circulating eosinophiles in every patient, in most instances to zero. This occurred on the first to the fourth day of ACTH administration, and on the fourth or fifth day of cortisone. Additional evidence of adrenal cortical stimulation by ACTH was provided by measurement in two patients (L. P. and E. C.) of the urinary excretion of 17-ketosteroids and 11-oxysteroids. The former increased from an average of 6.9 and 15.6 mg./24 hrs. before ACTH to 12.1 and 25.4 mg./24 hrs. during hormone administration, while the latter increased from an average of 0.14 and 0.35 mg./24 hrs. to 0.40 and 0.85 mg./24 hrs.

RESULTS

Effect of ACTH (Table II)

In four patients there was no change in basal strength or in neostigmine or tetraethyl pyrophosphate (TEPP) requirement or response during and following hormone administration. These patients included two with moderate weakness, one with severe, and one with very severe weakness. The latter patient (M. E.) received two courses of ACTH at an interval of two months, without any effect on her course. In the other six patients there was a slight to moderate decrease in strength and in response to neostigmine during ACTH administration. Four of these patients required additional neostigmine, which had to be administered intramuscularly to three patients (G. M., E. C., and E. W.) because of the onset of difficulty in swallowing. Two patients also began to have moderately severe weakness of the muscles of respiration. The decrease in strength was noted on the first to the fourth day of hormone administration,

TABLE I

Description of myasthenic patients. Severity of weakness classified as + (mild), ++ (moderate), +++ (severe), and ++++ (very severe)

PT.	AGE	SEX	WT.	DURATION OF MYASTHENIA	SEVERITY OF WEAKNESS		DRUG REQUIREMENT (mg.q. d.)			DESCRIPTION*			
					Bulbar	Peripheral	Neostigmine (o)	Neostigmine (i.m.)	TEPP (o)	Basal State	Grip Strength	Max. after Neostig. or TEPP	Grip Strength
	yrs.		*Kg.*	*Months*							*Kg.*		*Kg.*
M. B.†	24	F	47	6	++	++	None administered.			Facial and neck muscles weak. Voice nasal. Up and about, but with easy fatigue.	10	Near normal, except for easy fatigue.	24
L. K.	32	F	40	200	++	++			6	As above.	13	Near normal, except for easy fatigue.	27
L. P.	36	F	41	9	++	++	300			Facial and neck muscles weak. Unable to get out of bed.	15	Near normal, except for easy fatigue.	29
G. M.	19	F	64	4	+++	+++	240			Facial very weak. Swallowing difficult. Unable to raise head, arms, or legs, roll over, or sit up.	16	Ambulatory, but with easy fatigue.	28
G. R.	31	F	53	108	+++	+++	45		11.5	As above.	3	Near normal.	27
H. M.	56	M	64	4				12		As above.	2	Poor response to neostig. Could barely sit up.	9
A. M.	34	M	66	60	+	+++			11	Unable to raise head or extremities or sit up.	7	Near normal.	24
M. E.	42	F	45	15	++++	++++		24		Unable to swallow liquids, raise head, or	0	Poor response to neostig. Unable to swal-	4

M. E.	42	F	45	15	++++	++++		24		Unable to swallow liquids, raise head, or roll over.	0	Poor response to neostig. Unable to swallow liquids, raise head, or roll over. In respirator part of time.	4
E. C.	26	M	77	6	++	++++	450			Mod. facial weakness. Swallowing difficult. Unable to raise neck or arms or roll over.	0	Poor response to neostig. Could barely sit up.	15
E. W.	38	M	48	10	++	++++	90			Unable to raise head or extremities. Slight respiratory difficulty.	2	Able to get out of bed with help.	9
C. K.	12	F	58	2	+	++	67			Mod. facial weakness. Could barely raise head and arms. Flexors of legs weak. Gait shuffling.	14	Ambulatory, but with easy fatigue.	18
A. F.	32	F	57	18	+	++	240			Mod. weakness of face, arms, and legs, but ambulatory. Sl. difficulty swallowing.	12	Ambulatory, but with easy fatigue.	19
M. B.‡	25	F	47	187	++	++++	315			Mod. weakness of face, neck and arms. Voice nasal. Could barely raise leg. Unable to sit up or get out of bed.	9	Ambulatory, but with easy fatigue.	13

* All patients also had ptosis and limitation of extraocular movement, with diplopia, in the basal state. In most patients the latter was not restored to normal following neostigmine or TEPP.

† First admission, for ACTH administration.

‡ Second admission, for cortisone administration.

TABLE II

Effect of ACTH or cortisone administration on strength and neostigmine or TEPP requirement of 12 myasthenic patients

PT.	ACTH		KCl Gm. q.d. (o)	CHANGE IN:				SINCE ACTH OR CORT.	
				Basal and Max. Strength		Daily Neostigm. or TEPP Requirement			
	mg.q.d. i.m. (Av.)	Days Total		During ACTH	After ACTH	During ACTH	After ACTH	Months After	Course
				(days in parentheses)					
M. B.	112	11	6	mod. weaker (4–11)	n.c. (no change)	incr. to 105 mg. o.	incr. to 135 mg. o.	12	weaker; neostig. req. incr. to 315 mg. o.
L. K.	80	5	0	n.c.	n.c.	n.c.	n.c.	16	n.c.
L. P.	40	4	0	n.c.	n.c.	n.c.	n.c.	34	n.c.
G. M.	100	5	3	sl. weaker (3–5)	n.c.	incr. by 2 mg. i.m.	n.c.	18	n.c.
G. R.	80	5	3	n.c.	n.c.	n.c.	n.c.	16	n.c.
H. M.	80	5	3	sl. weaker (3)	n.c.	n.c.	n.c.	16	n.c.
A. M.	80	5	0	mod. weaker (2–5)	mod. weaker (1)	n.c.	n.c.	16	n.c.
M. E.	80	5	4	n.c.	n.c.	n.c.	n.c.	19	n.c.
	80	6	0	n.c.	n.c.	n.c.	n.c.	17	n.c.
E. C.	80	4	0	mod. weaker (4)	mod. weaker (1–7)	incr. by 6 mg. i.m.	incr. by 2–8 mg. i.m.	8	died, resp. failure.
E. W.	100	3	0	mod. weaker (1–3)	much weaker (1–20)	incr. to 195 mg. o. + 4.5 mg.i.m.	incr. to 24 mg. i.m.	9	died, resp. failure.
	CORTISONE								
C. K.	150	11	4.5	mod. weaker (9–12)	mod. weaker (1–5)	incr. to 202 mg. o.	incr. to 240 mg. o. + 1 mg.i.m.	11	n.c.
A. F.	200	12	6	sl. weaker (11–12)	mod. weaker (1–6)	n.c.	incr. to 12 mg. i.m.	12	much weaker
M. B.	200	8	3	mod. weaker (5–8)	much weaker (1–30)	incr. by 1.5 mg. i.m.	incr. to 405 mg. o. + 3 mg.i.m.	2	much weaker

and in most instances continued administration resulted in some further diminution in strength.

Following the cessation of ACTH, the four patients who had shown no change continued to be unaltered. Three patients who had become weaker returned to their initial level of strength and of neostigmine response and requirement within one to two days after the last dose of ACTH. During the 16 to 34 months following hormone administration that these seven patients have been observed, there has been no appreciable change. One patient (M. B.) remained at the same level of diminished strength for several weeks after ACTH and in the ensuing 12 month period had a further diminution in strength and increase in neostigmine requirement. One patient (E. C.) remained moderately weaker for one week after ACTH, and continued to require larger doses of neostigmine by mouth, as well as several hypodermic injections each day, because of difficulty in swallowing and breathing. His strength and neostigmine requirement then returned to their initial level for a period of eight months, following which there was an exacerbation of the disease and death occurred in respiratory failure. Another patient (E. W.), who had been growing gradually weaker prior to hormone administration, became worse even more rapidly during ACTH, which was discontinued after only three days of administration. On the third day he received large amounts of neostigmine by mouth and required several intramuscular injections because of respiratory weakness. On the following day the injection of 2 mg. of neostigmine every two hours was insufficient to maintain respiration, and he had to be placed in a respirator. He remained critically ill, requiring continuous respirator care, for a month, following which his strength gradually improved and his neostigmine requirement decreased, though not to the level that had existed prior to ACTH. Nine months after hormone administration there was an exacerbation of weakness, and he died in respiratory failure.

A. F.	200	12	6	sl. weaker (11–12)	mod. weaker (1–6)	n.c.	incr. to 12 mg. i.m.	12	much weaker
M. B.	200	8	3	mod. weaker (5–8)	much weaker (1–30)	incr. by 1.5 mg. i.m.	incr. to 405 mg. o. + 3 mg. i.m.	2	much weaker

Effect of Cortisone (Table II)

One patient (A. F.) became slightly weaker on the eleventh and twelfth days of cortisone administration. During the week following cessation of hormone, there was a further decrease in strength and in response to neostigmine. The patient became unable to swallow, sit up, or raise her arms, and she required intramuscular injections of neostigmine every two or three hours. Following this period there was a gradual increase in strength and in neostigmine response, to the pre-cortisone level. Twenty-five days after cessation of cortisone, however, there was a return of severe weakness, requiring intramuscular injections of neostigmine for the relief of impaired swallowing and respiration. After one week the patient had to be placed in a respirator, where she remained almost continuously for a period of five months. There was then a gradual in-

crease in strength and neostigmine response, so that oral neostigmine could be resumed, but during the ensuing six months the myasthenia remained considerably more severe than prior to cortisone.

Patient C. K. became moderately weaker on the eighth through the eleventh day of cortisone administration, with increased weakness of the muscles of the face, jaws, and extremities, and onset of dysphagia. The neostigmine requirement increased threefold. Following cessation of cortisone the increased weakness persisted for five days, during which time the oral neostigmine requirement increased further, and several intramuscular injections of neostigmine had to be administered because of difficulty in swallowing and breathing. After this period the strength and neostigmine response gradually increased, but these had not returned to the pre-cortisone level twenty-five days after the cessation of hormone, at which time the neostigmine requirement was twice that prior to cortisone. During the ensuing ten months there was a gradual improvement in strength, to about the pre-cortisone level.

Patient M. B. became moderately weaker on the fifth through the eighth day of cortisone administration, with increased weakness of the extremities and the onset of dysphagia. On the sixth day of hormone administration a course of daily X-ray irradiation of the thymic region was begun, and 2,000R were administered over nine days. Following cessation of cortisone there was a further decrease in strength and in response to neostigmine. The patient was barely able to move her extremities, in spite of large oral doses of neostigmine, supplemented by parenteral administration. On the fourth and fifth days after the last injection of cortisone there was a transient alteration in the response to large doses of neostigmine, which produced an increase in strength (though not to the level that had existed prior to cortisone), followed by generalized fasciculations and marked weakness. There was also an increase in the muscarine-like effects of neostigmine (sweating, salivation, abdominal cramps, nausea, cardiospasm, and diarrhea), which necessitated an increase in the intake of atropine sulfate from one to 4.6 mg. a day. The increased atropine requirement persisted during the following two weeks, while the response of the voluntary muscles to neostigmine remained very poor. There was then a very gradual increase in strength and neostigmine response, but these were still considerably below the pre-cortisone level two months after the cessation of hormone.

The increase in weakness that occurred during and after cortisone administration was not associated with evidence of suppression of adrenal cortical activity in any of the three patients studied. There was no significant depression of the daily urinary excretion of 17-ketosteroids or 11-oxysteroids, and no alteration in the serum concentration of sodium or potassium, or in the blood pressure.

Serum Concentration of Potassium and Effect of Potassium Administration

Seven of the twelve patients received three to six grams of potassium chloride by mouth daily during ACTH or cortisone administration (Table II), and their dietary intake of sodium chloride was limited to four grams a day. One patient received potassium chloride during her first course of ACTH, but not during the second. The incidence and severity of increased weakness during or following hormone administration was no different in these patients than in those who did not receive potassium chloride or dietary restriction of sodium. The oral administration of two to four grams of potassium chloride during the period of increased weakness had no discernable effect on muscle strength or neostigmine response. The serum concentration of potassium was not significantly altered either during or after hormone administration in four patients in whom this was determined (M. B. during ACTH and cortisone, L. P., E. W., and C. K.), including two who received no potassium chloride during their brief period of adrenal cortical stimulation.

Effect of Thymectomy and of Irradiation of Thymus Following ACTH or Cortisone

None of the patients who was studied had roentgenographically demonstrable enlargement of the thymus. Two patients (H. M. and E. C.) were subjected to thymectomy 8 and 30 days after receiving ACTH. One patient (M. B.) had irradiation of the thymic region during and after cortisone administration, while another (C. K.) had irradiation (2,800R over 12 days) beginning 12 days after cessation of cortisone. In none of these patients was there any improvement during or after these measures.

ONSET OF MYASTHENIA GRAVIS DURING PROLONGED CORTISONE ADMINISTRATION

J. H. H. #576665. B. W., a 65 year old white woman, had had rheumatoid arthritis since the age of 25, with crippling deformities of almost all joints since the age of 50. She was almost bedridden. There was marked limitation of motion and moderately severe pain in most of the joints, and some atrophy and weakness of the muscles of the extremities attributable to the rheumatoid arthritis. There was no weakness or abnormal fatigability of the muscles innervated by the cranial nerves. During a period of eight months prior to hospital admission the patient received cortisone daily, for the amelioration of her rheumatoid arthritis. She received 300, 200, and then 100 mg. a day intramuscularly for two weeks, followed by 50 mg. a day intramuscularly for 5½ months. She then received 50 mg. a day orally for 2 months. During the eight months of cortisone administration she was given two grams of potassium chloride by mouth daily, and her dietary intake of sodium chloride was limited to approximately four

grams a day. Shortly after the onset of cortisone therapy the joint pains diminished markedly, and remained very mild. The patient discontinued taking aspirin, which she had been consuming in large amounts. Motion of the joints did not improve. There was increased appetite, weight gain of 13 pounds, and an increase in alertness, subjective strength, and feeling of well being. There may have been slight euphoria. No other side effects of cortisone were noted.

During the seventh month of cortisone administration, the patient began to have slight difficulty swallowing. During the eighth month she developed a transient upper respiratory infection, with cough, malaise, and sore throat. Swallowing became more difficult and cortisone was discontinued. During the following week severe generalized weakness and abnormal fatigability appeared. The tongue felt thick and speech was slurred. The muscles of mastication fatigued rapidly. There was difficulty holding the head up. The arms and legs could be raised for only a few seconds.

The intramuscular injection of 1.5 mg. of neostigmine (with 0.6 mg. atropine) was followed within 45 minutes by marked improvement in general strength, including swallowing. There was also improvement following the oral administration of 15 mg. of neostigmine every two hours, but within a week the response to neostigmine diminished, and the patient had difficulty swallowing, with choking spells, despite the intramuscular injection of 0.5 mg. of neostigmine every two hours.

Physical examination at the time of admission to the hospital three weeks after the cessation of cortisone administration revealed severe impairment of swallowing and moderate generalized weakness. There was marked weakness of the facial muscles, including the orbicularis oculi. There was moderate ptosis, which increased with fatigue of the levators. Speech was nasal and slurred and the lower jaw sagged. There was moderate weakness of the muscles of the neck, trunk, and extremities. There was marked deformity and limitation of motion of almost all the joints, with ankylosis of the wrists and ankles, marked flexion deformity of the hands and ulnar deviation and fixed flexion of the fingers. The muscles of the hands, forearms, deltoids and thighs were moderately wasted. The blood pressure was 200/94 mm. of mercury. The remainder of the physical examination was not remarkable.

Examination of the formed elements of the blood was normal except for the sedimentation rate (28 mm./hour, corrected). The eosinophile count varied between 110 and 264/mm^3. The serum concentration of sodium was 142 meq./l., of potassium 3.6 meq./l., and of cholesterol 237 mg. per cent. Determinations of basal metabolic rate were +18 and −3. The urinary excretion of 17-ketosteroids was low, being 1 mg./24 hours.

At the time of admission to the hospital, severe impairment of swallowing necessitated the intramuscular injection of neostigmine at two hour intervals.

The oral administration of potassium chloride, in doses of two grams, had no effect on muscular strength or neostigmine requirement. During the ensuing month there was a gradual increase in strength and in response to neostigmine, so that the patient could be maintained on oral neostigmine, except for one intramuscular injection a day. During the following five months there was a further gradual increase in strength and decrease in neostigmine requirement. Coincident with the patient's improvement there was a progressive increase in urinary excretion of 17-ketosteroids (Table III). The urinary excretion of 11-oxysteroids, which was not determined until seven weeks after the cessation of cortisone, was normal at that time. A course of irradiation to the anterior mediastinum (2,000R over ten days) was administered nineteen weeks after cessation of cortisone, at which time moderate clinical improvement and increase in 17-ketosteroid excretion had occurred.

TABLE III

Urinary excretion of 17-keto and 11-oxysteroids, neostigmine requirement, and degree of weakness of patient B. W.

DAY AFTER CESSATION OF CORTISONE	URINARY 17-KETOSTEROIDS MG./24 HRS.	URINARY 11-OXYSTEROIDS MG./24 HRS.	NEOSTIGMINE REQUIREMENT MG. Q.D.		WEAKNESS	
			i.m.	o.	Bulbar	Peripheral
30	1.0		12.5	—	++++	+++
34	1.8		6.5	150	+++	++
50	2.3	0.9	1	240	++	++
66	3.4	1.2	1	240	++	++
104	3.6	0.5	0	240	++	++
120	4.4	0.5	0	225	++	+
136	5.7	0.4	0	195	++	+
224	4.7	0.8	0	150	++	+

DISCUSSION

The prolonged administration of ACTH or cortisone to non-myasthenic subjects is occasionally associated with the development of generalized muscular weakness (9, 10), which may or may not be associated with depression of the serum concentration of potassium. Most of the patients with myasthenia gravis who were studied developed a decrease in strength and in neostigmine response following much shorter periods of administration of these hormones. The mechanism of this exacerbation of myasthenia gravis is not known. It was not prevented or ameliorated by the administration of potassium chloride, nor was it associated with lowering of the serum concentration of potassium, through the possibility of lowered intracellular concentration of potassium cannot be excluded. It was not accompanied in the myasthenic subjects by any evidence of decreased adrenal cortical activity. The patients who received

ACTH showed evidence of adrenal cortical stimulation, as indicated by the number of circulating eosinophiles and the urinary excretion of 17-ketosteroids and 11-oxysteroids, as well as by other metabolic changes that were studied (12). In view of the absence of any observed improvement in strength or neostigmine requirement following ACTH or cortisone administration, and in view of the serious exacerbation that may occur, these hormones would appear to have no role in the management of myasthenia gravis.

The development of myasthenia gravis during prolonged administration of cortisone to a non-myasthenic subject is of considerable interest, particularly since the incidence of onset of this disease in women past the age of 60 is very low (13). It is not uncommon for myasthenia gravis to begin at this age in men, but of more than 100 women with this disease who have been admitted to the Johns Hopkins Hospital, in no other patient did the initial symptoms begin after the age of 60. It, therefore, appears possible that the onset of myasthenia gravis during prolonged cortisone administration may have been more than a coincidence, even though this association has not been observed in numbers of other subjects who have received protracted courses of cortisone. The patient who was studied had evidence of reduced adrenal cortical activity thirty days after the cessation of cortisone, as reflected by the low excretion of 17-ketosteroids, but the normal serum concentration of sodium and potassium, and the elevated blood pressure, indicate that the reduction in adrenal cortical activity was not of a profound degree. The weakness could not be ascribed to adrenal insufficiency alone, since the weakness of patients with Addison's disease is not ameliorated by neostigmine. However, since improvement of the patient's myasthenia coincided with a gradual increase in urinary 17-ketosteroid excretion, it is possible that the onset and severity of the disease may have been related to the action of cortisone or to suppression of adrenal cortical activity. Although onset of myasthenia gravis, as occurred in this patient, must be an uncommon cause of the weakness that may occur during ACTH or cortisone administration to non-myasthenic subjects, it would appear desirable to consider this possibility when weakness occurs, and to observe the effect of neostigmine upon it.

The relation of myasthenia gravis to the glands of internal secretion is not known. However, the severity of the disease can be affected by the administration or withdrawal of a number of hormones, and by physiologic states associated with changes in endocrine function. Myasthenic women almost invariably become weaker for several days prior to the onset of each menstrual period, and improve during the menses (13). It is not uncommon for myasthenia gravis to improve during pregnancy, particularly during the latter months (14). In patients with hyperthyroidism, the incidence of myasthenia gravis is higher than would be expected from chance association, and, when the two diseases

are associated, treatment of the hyperthyroidism is usually followed by improvement in the myasthenia gravis (15). The concurrence of Addison's disease and myasthenia gravis has been reported (16), but is apparently rare. The exacerbation of myasthenia gravis which may follow the administration of ACTH or cortisone, and the observation of onset of myasthenia gravis during prolonged cortisone administration, provide additional illustrations of the influence of endocrine substances on this disease.

SUMMARY

The administration of ACTH or cortisone produced a decrease in strength and in response to neostigmine in eight of twelve patients with myasthenia gravis. This effect was not accompanied by any alteration of the serum concentration of potassium, nor was it prevented or ameliorated by the administration of potassium chloride. Following the cessation of hormone administration there was a further decrease in strength and neostigmine response in five patients, lasting one to four weeks. In no patient was there any significant improvement in the myasthenia gravis either during or following hormone administration.

Myasthenia gravis was observed to begin during the seventh month of administration of cortisone to a patient with rheumatoid arthritis, and to become severe following cessation of the hormone. This was accompanied by a prolonged reduction in urinary 17-ketosteroid excretion. During the ensuing five months there was a gradual and moderate improvement in the myasthenia, and an increase in 17-ketosteroid excretion.

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STUDIES OF α-KETOGLUTARIC ACID OXIDASE ACTIVITY IN WASHED RESIDUES OF THE MAMMALIAN KIDNEY*

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Working with homogenates prepared in isotonic potassium chloride, Ackermann (1) has developed a manometric method for the assay of α-ketoglutaric acid oxidase activity in mammalian tissues. His complete system contains magnesium, phosphate, adenosine triphosphate, cytochrome *c*, diphosphopyridine nucleotide (DPN), thiamine pyrophosphate (cocarboxylase), malonate, α-ketoglutarate and tissue. Holding the malonate concentration uniformly at 3×10^{-3}M, he determined the optimal concentrations for all other components in terms of oxygen uptake. From the close parallel between oxygen uptake and α-ketoglutarate disappearance, he concludes that oxygen utilization with his system is a measure of the rate at which various tissues oxidize α-ketoglutarate; and that DPN is required for the oxidation (at maximal rates) of α-ketoglutarate by liver and brain homogenates.

In exploring the applicability of Ackermann's method to the assay of α-ketoglutaric acid oxidase activity in washed residues of the mammalian kidney, we were led by a variety of experiments to examine more closely the possibilities of assay in the absence of malonate, with the system operating at maximal rates. Such a procedure foregoes the advantages, for certain purposes, of isolating the conversion of α-ketoglutarate to succinate from the remainder of the tricarboxylic acid cycle. However, this is unimportant if one is interested primarily in evaluating levels of enzyme activity. The experimental data indicate that α-ketoglutaric acid oxidase can be satisfactorily assayed under these conditions in washed residues of the rabbit kidney.

MATERIAL AND METHODS

Adenylic acid was obtained from Ernst Bischoff Co., adenosine triphosphate (sodium salt) from Armour Laboratories, diphosphopyridine nucleotide (DPN) from Schwartz Laboratories, and α-ketoglutaric acid, cytochrome *c* and thiamine pyrophosphate (cocarboxylase) from Nutritional Biochemicals Corp. The sample of DPN had a rated purity of 84.7% based on assay by the method of Gutcho and Stewart (2), using the molecular extinction coefficient of 6.2×10^6 recommended by Horecker and Kornberg (3). Correction for purity was made in preparing solutions for use. The concentration of cytochrome *c* solutions was determined with the Beckman spectrophotometer, using a modification

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(details of which were kindly supplied by Wyeth, Inc.) of the method of Rosenthal and Drabkin (4). From the analytical data supplied, the sample of cocarboxylase was of a high degree of purity, and correction was made only for water content. Solutions of adenylic and α-ketoglutaric acids were routinely adjusted to pH 7.3 prior to use.

Homogenates and washed residues of the rabbit kidney were prepared by a method differing only in details from that of Green *et al.* (5). Two rabbits (c. 2 kg. each) were stunned

TABLE I

Oxygen Utilization by Mouse Liver, Homogenized in 0.9% KCl-0.01M PO_4 Buffer, and Assayed with α-Ketoglutarate in Ackermann's System

EXPT. NO.	TISSUE PER VESSEL	OXYGEN UTILIZATION					
		Cumulative			Per 20 mg. tissue per 10 min.		
		10 min.	20 min.	30 min.	0–10 min.	10–20 min.	20–30 min.
	mg	μl	μl	μl	μl	μl	μl
1	20	7	13	14	7.0	6.0	1.0
	40	26	51	67	13.0	12.5	8.0
	60	48	98	138	16.0	16.7	13.3
2	20	8	11	10	8.0	3.0	0
	40	22	43	58	11.0	10.5	7.5
	60	40	86	127	13.3	15.3	13.7
3	20	11	14	15	11.0	3.0	1.0
	40	31	61	81	15.5	15.0	10.0
	60	53	111	163	17.7	19.3	17.3
4	20	4	6	5	4.0	2.0	0
	40	24	50	72	12.0	13.0	11.0
	60	62	125	180	20.7	21.0	18.3
5	20	1	3	3	1.0	2.0	0
	40	29	57	79	14.5	14.0	11.0
	60	55	117	173	18.3	20.7	18.7

Each vessel contained 0.1 ml. 0.05M $MgCl_2$, 0.2 ml. 0.1M PO_4 buffer pH 7.3, 0.3 ml. 0.01M adenosine triphosphate, 0.1 ml. 4×10^{-4}M cytochrome *c*, 0.1 ml. each of 0.05% DPN and 0.05% cocarboxylase, 0.1 ml. 0.09M malonate, 27.2 μM α-ketoglutarate, and 0.4, 0.8 or 1.2 ml. 5% mouse liver homogenate. All components at optimal final concentrations as reported by Ackermann (1). Homogenates prepared in all-glass apparatus of Potter-Elvehjem type (with beads on pestle) for expts. 1 and 2, of Memorial type (without beads on pestle) for expts. 3–5. Temperature—38°. Equilibration period 5 min. All homogenates passed through 0.5 mm. sieve before use.

by a blow on the head and bled from the neck vessels. The kidneys were removed, stripped of fat and placed in ice. Within 10 minutes or less they were homogenized with 240 ml. of cold (c. 4° C.) 0.9% KCl-0.01M PO_4 buffer pH 7.3 in a Waring blendor (vessel pre-chilled in deep freeze) for 2 minutes. One drop of 6N NaOH was added to the KCl-buffer at the beginning, and during homogenization sufficient N NaOH was added to maintain the pH at c. 7.0 (tested at frequent intervals with bromthymol blue). The homogenate was passed through a 0.5 mm. sieve to remove connective tissue, etc. Homogenization and sieving were accomplished in a cold room (3–4°); all subsequent procedures were accomplished in

a refrigerated centrifuge (International PR-1, set to give a maximum temperature of c. 4° in the tube contents) or in an ice-water bath. The homogenate was divided between 3 100 ml. lusteroid cups and centrifuged 15 minutes at 3000 r.p.m. (conical head). The supernatant was discarded and the residue (R_1) resuspended in 240 ml. of cold KCl-buffer and centrifuged as before. The second residue (R_2) was treated like the first. At the R_3 stage the residues from the 3 tubes were combined, and the tubes were washed with sufficient KCl-buffer (usually c. 3 ml.) to bring the final suspension, after thorough mixing, to a rather thick and smooth consistency. The volume of this "thick" residue was usually c. 20 ml. For present purposes we employed routinely a "dilute" residue, prepared by diluting the thick residue with an equal volume of KCl-buffer.

TABLE II

Oxidation of 30 μM. of α-Ketoglutarate in the Presence of 100 μM. of Malonate, with and without Adenylate and with Varying Amounts of Rabbit Kidney Residue

EXPT. NO.	KIDNEY RESIDUE PER VESSEL	OXYGEN UTILIZATION			
		With adenylate		Without adenylate	
		5 min.	90 min.	5 min.	90 min.
	ml	μl	μl	μl	μl
1A	0.25	22	117	—	—
	0.5	54	365	—	—
Fresh	1.0	103	529	—	—
1B *4 hrs. old*	1.0	67	458	35	91
2 *Fresh*	1.0	92	547	58	219

Theoretical O_2 requirement for total conversion of 30 μM. α-ketoglutarate to succinate, 336 μliters.

Each vessel contained 0.2 ml. 0.02M $MgCl_2$, 0.5 ml. 0.04M PO_4 buffer pH 7.3, 0.3 ml. 0.01M Na adenylate (omitted as indicated), 0.1 ml. M malonate, 0.3 ml. 0.1M α-ketoglutarate, the indicated amount of "thick" residue, and water. α-Ketoglutarate placed in sidearm of iced vessels, and added to main compartment after gassing had been completed and just before vessels entered 37° bath. Oxygen utilization during first 5 minutes recorded to allow correction for 5 minute equilibration period.

The "granule fraction" used for expts. 8 and 9, Table III was prepared as above through the resuspension of R_2, which was then centrifuged 5 minutes at 1000 r.p.m. The supernatant and the upper layer (which pours off easily) of the residue (R_3) were decanted, mixed and centrifuged 5 minutes at 1000 r.p.m. The supernatant and upper layer of the residue (R_4) were again decanted, mixed and centrifuged, this time for 15 minutes at 3000 r.p.m. The final residues (R_5) from all tubes were combined and thoroughly mixed to give a moderately thick, creamy suspension, which was diluted with 2 volumes of KCl-buffer before use.

The experiments were carried out in conventional Warburg equipment, and the following conditions apply unless otherwise specified: reaction volume—3.0 ml.; homogenates and residues added to vessels last, immediately after their preparation was completed, and promptly mixed thoroughly with vessel contents; vessels kept in ice-water bath up to the

time of gassing (2 minutes with frequent shaking); gas phase—pure oxygen; NaOH in center well; temperature—37°; rate—120 strokes per minute (3 cm. stroke). Control vessels (without added substrate, but components otherwise identical with the experimental system) were included in all experiments, and their rates of oxygen utilization have been subtracted from those of the experimental vessels to give the figures reported in the accompanying tables and figures. With residues and granule fractions of the kidney oxygen utilization in the control vessels is routinely quite low; with homogenates, as is well known, it is rather high. In the course of this and other studies the manometer fluid recommended by Krebs (6) has been given a thorough trial, and has proved highly satisfactory.

TABLE III

Malonate Inhibition of α-Ketoglutarate Oxidation as a Function of Substrate/Inhibitor Ratio, at Two Levels of Substrate Concentration, in the Presence of a Washed Residue of the Rabbit Kidney

SUBSTRATE / INHIBITOR RATIO	10 μM. α-KETOGLUTARATE*						20 μM. α-KETOGLUTARATE†					
	Malonate		Oxygen utilization				Malonate		Oxygen utilization			
			5 Min.	60 Min.	5 Min.	60 Min.			5 Min.	60 Min.	5 Min.	60 Min.
	μM	μM	μl	μl	μl	μl	μM	μM	μl	μl	μl	μl
2:1	5	—	78	384	—	—	10	—	104	652	—	—
1:1	10	—	79	293	—	—	20	—	88	443	—	—
1:2	20	20	58	191	61	197	40	40	68	349	56	316
1:3	30	30	62	173	56	179	60	60	65	309	52	272
1:4	—	40	—	—	46	158	—	80	—	—	43	237
1:5	—	50	—	—	47	141	—	100	—	—	35	198
1:6	—	60	—	—	40	131	—	120	—	—	30	160

* Theoretical O_2 requirement for total conversion to succinate, 112 μliters.

† Theoretical O_2 requirement for total conversion to succinate, 224 μliters.

Each vessel contained 0.2 ml. 0.02M $MgCl_2$, 0.5 ml. 0.04M PO_4 buffer pH 7.3, 0.3 ml. 0.01M Na adenylate, the indicated amounts of malonate and α-ketoglutarate, 0.5 ml. "thick" residue, and water. α-Ketoglutarate placed in sidearm of iced vessels, and added to main compartment after gassing had been completed and just before vessels entered 37° bath. Studies accomplished by 2 individuals as rapidly as possible, using same residue throughout. Two experimental runs at higher substrate/inhibitor ratios (2:1 to 1:3), with 10 and 20 μM. of α-ketoglutarate, completed prior to overlapping runs at lower ratios (1:2 to 1:6). In all experiments oxygen utilization had either virtually ceased by 60 min., or was proceeding too slowly to alter interpretation of results. Oxygen utilization during first 5 minutes recorded to allow correction for 5 minute equilibration period. In each of the 4 experimental runs control vessel (without substrate) contained largest amount of malonate used in that particular run.

In studies of α-ketoglutarate disappearance the requisite number of experimental and control (without added substrate) vessels were prepared, gassed, and equilibrated together, and removed from the bath at the desired intervals. Vessel contents were promptly poured into a solution of trichloracetic acid (final concentration 5% in the early experiments, 8½% in the remainder), and the vessel was rinsed with two 2 ml. portions of water. In determining α-ketoglutarate disappearance as a function of time, at several intervals in the same experiment, a single control vessel served for the entire experimental series, and its contents were transferred to trichloracetic acid at the end.

α-Ketoglutaric acid was determined by the Friedemann (7) modification of the method of Friedemann and Haugen (8), using the Beckman spectrophotometer and determining

TABLE IV

Oxygen Utilization and α-Ketoglutarate Disappearance in the Presence of Cytochrome c ($0.7 \times 10^{-5}M$), in Experiments with Blendor Homogenates of the Rabbit Kidney

EXPT. NO.	TISSUE PER VESSEL	NITROGEN PER VESSEL	EXPERIMENTAL PERIOD	α-KETOGLUTARATE			OXYGEN UTILIZATION
				At 0 min.	At end	Disappearance	
	mg	*mg*	*min*	*μM*	*μM*	*μM*	*μM*
1	35	0.87	20	39.6	37.0	2.6	3.2
	70	1.74	20	37.3	34.0	3.3	8.0
2	105	2.6	20	35.0	30.3	4.7	9.7
	210	5.2	20	31.5	21.7	9.8	21.8
3	210	5.2	10	31.2	23.4	7.8	12.8
	210	5.2	20	31.2	17.5	13.7	26.4
	210	5.2	30	31.2	12.6	18.6	38.8
	210	5.2	40	31.2	9.1	22.1	51.1

α-Ketoglutarate, initial, 41 μM. Each vessel contained 0.7 or 1.4 ml. homogenate (expt. 3, 1.4 ml.) and 0.15 ml. 1.4×10^{-4}M cytochrome *c*. Strength of homogenate 5% in expt. 1, 15% in expts. 2 and 3. Equilibration period 10 min.

The following basic additions were made to the vessels in all experiments recorded in Tables IV–XI: 0.2 ml. 0.02M $MgCl_2$, 0.4 ml. 0.04M PO_4 buffer pH 7.3, 0.3 ml. 0.01M Na adenylate, and water as required to bring the reaction volume to 3.0 ml.

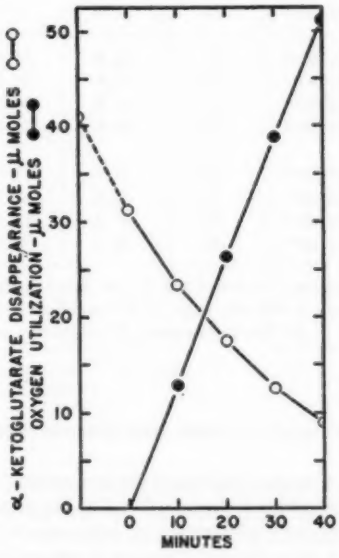

FIG. 1. Chart of expt. 3, Table IV

the optical density at 420 millimicrons. The calibration curve was constructed with α-ketoglutaric acid twice recrystallized (acetone-benzene, melting point 112.5–114.5°) from a sample obtained from Nutritional Biochemicals Corp. Determinations were made routinely at an optical density in the neighborhood of 0.200, and all analyses were accomplished in duplicate. The amount of α-ketoglutarate added to the vessels was determined for all

TABLE V

Oxygen Utilization and α-Ketoglutarate Disappearance as a Function of Time in Experiments with Washed Residues of the Rabbit Kidney

EXPT. NO.	NITROGEN PER VESSEL	ADDED CYTOCHROME *c*, FINAL CONCENTRATION	EXPERIMENTAL PERIOD	α-KETOGLUTARATE			OXYGEN UTILIZATION
				At 0 min.	At end	Disappearance	
	mg	*molarity*	*min*	*μM*	*μM*	*μM*	*μM*
1	2.40	—	10	23.8	16.0	7.8	8.1
	2.40	—	20	23.8	10.1	13.7	16.3
	2.40	—	30	23.8	6.2	17.4	23.6
	2.40	—	40	23.8	2.6	21.2	30.5
2	1.42	0.7×10^{-5}	10	34.8	28.5	6.3	7.8
	1.42	0.7×10^{-5}	20	34.8	24.0	10.8	15.6
	1.42	0.7×10^{-5}	30	34.8	19.5	15.3	21.4
	1.42	0.7×10^{-5}	40	34.8	16.2	18.6	27.3
	2.83	0.7×10^{-5}	10	27.0	16.5	10.5	16.9
	2.83	0.7×10^{-5}	20	27.0	8.7	18.3	30.8
	2.83	0.7×10^{-5}	30	27.0	4.2	22.8*	42.0*
	2.83	0.7×10^{-5}	40	27.0	1.8	25.2*	50.8*
3	0.95	0.7×10^{-5}	10	36.9	33.0	3.9	4.8
	0.95	0.7×10^{-5}	20	36.9	28.8	8.1	9.5
	0.95	0.7×10^{-5}	30	36.9	26.4	10.5	13.4
	0.95	0.7×10^{-5}	40	36.9	23.8	13.1	16.9
	1.90	0.7×10^{-5}	10	32.4	23.8	8.6	10.6
	1.90	0.7×10^{-5}	20	32.4	18.2	14.2	20.0
	1.90	0.7×10^{-5}	30	32.4	12.5	19.9	28.4
	1.90	0.7×10^{-5}	40	32.4	9.6	22.8	34.8

α-Ketoglutarate, initial, 28.6 (expt. 1) or 40.3 μM. (expts. 2 and 3). Each vessel contained varying amounts of kidney residue (expt. 1—0.5 ml., expt. 2—0.3 or 0.6 ml., expt. 3—0.2 or 0.4 ml.) and, in expts. 2 and 3, 0.15 ml. 1.4×10^{-4}M cytochrome *c*. Equilibration period 5 min. in expt. 1, 10 min. in expts. 2 and 3.

* Not included in Figure 4.

experiments by analysis of vessel contents. Nitrogen was determined by the Kjeldahl procedure.

The oxidative properties of washed residues of the type utilized in this study are routinely studied in the presence of phosphate buffer of pH c. 7.3, and of added magnesium ions and muscle adenylic acid (adenosine-5-phosphate) or adenosine triphosphate (5, 9). These components have been used throughout the present investigation, at final concentrations

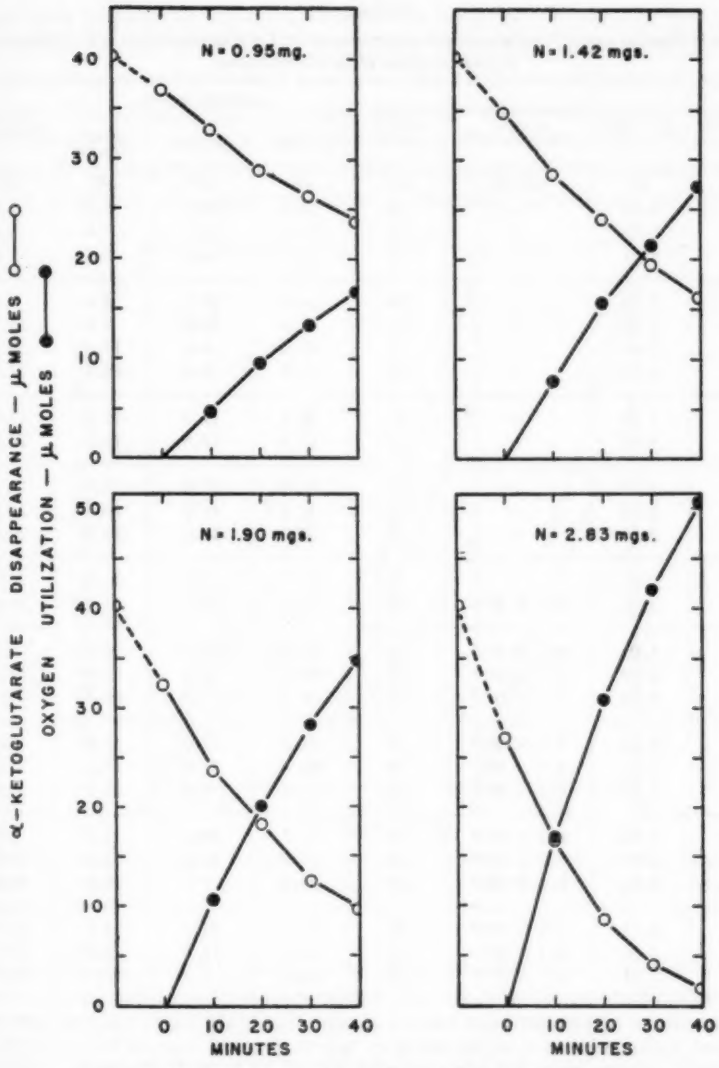

FIG. 2. Charts of expts. 2 (to right) and 3 (to left), Table V

which are well established as within the optimal range for the experimental system. Cytochrome *c* is frequently omitted, but its desirability as a component in studies of α-ketoglutaric acid oxidase activity has been demonstrated by the work of Ochoa (10), Ackermann (1) and others, and is confirmed by our data.

TABLE VI

Oxygen Utilization and α-Ketoglutarate Disappearance under Varying Conditions in Experiments with Washed Residues of the Rabbit Kidney

EXPT. NO.	NITROGEN PER VESSEL	ADDED CYTOCHROME *c*, FINAL CONCENTRATION	EXPERIMENTAL PERIOD	α-KETOGLUTARATE			OXYGEN UTILIZATION
				At 0 min	At end	Disappearance	
	mg	*molarity*	*min*	*μM*	*μM*	*μM*	*μM*
1	1.17	—	10	26.8	24.5	2.3	1.8
	2.18	—	10	25.1	21.7	3.4	4.8
	4.21	—	10	22.5	14.4	8.1	9.4
2	1.26	—	20	26.0	21.4	4.6	5.8
	2.35	—	20	23.4	14.0	9.4	13.2
	3.44	—	20	21.6	4.4	17.2	22.7
	4.53	—	20	19.9	0.6	19.3	31.2
3	2.26	—	20	31.4	19.2	12.2	17.9
	3.02	—	20	29.5	12.6	16.9	24.8
4	1.43	—	20	36.0	27.0	9.0	10.2
	2.14	—	20	32.4	21.7	10.7	16.2
	2.86	—	20	31.0	15.0	16.0	22.6
5	2.51	—	20	28.2	14.9	13.3	16.2
	2.51	0.7×10^{-5}	20	27.7	12.8	14.9	20.0
6	1.03	0.7×10^{-5}	20	38.5	32.0	6.5	7.5
	1.89	0.7×10^{-5}	20	35.0	22.1	12.9	15.8
	2.74	0.7×10^{-5}	20	31.5	13.0	18.5	24.2
7	1.42	0.7×10^{-5}	20	34.8	22.9	11.9	14.6
	2.10	0.7×10^{-5}	20	30.8	15.0	15.8	22.5
	2.75	0.7×10^{-5}	20	27.9	7.4	20.5	30.0
8*	0.74	0.7×10^{-5}	20	37.7	30.5	7.2	7.9
	1.36	0.7×10^{-5}	20	31.8	17.5	14.3	19.7
	1.97	0.7×10^{-5}	20	27.8	7.7	20.1	28.4
9*	1.17	0.7×10^{-5}	20	33.6	22.4	11.2	15.0
	1.76	0.7×10^{-5}	20	30.2	14.0	16.2	23.1
	2.34	0.7×10^{-5}	20	26.1	7.0	19.1	31.0

α-Ketoglutarate, initial, 28.6 (expts. 1 and 2), 31 (expt. 5) or 40 μM. (expts. 3,4,6–9). Each vessel contained varying amounts of kidney residue or "granule fraction" (between 0.2 and 1.0 ml., as indicated by nitrogen values) and, where indicated, 0.15 ml. 1.4×10^{-4}M cytochrome *c*. Equilibration period 10 min. in all except expts. 1, 2 and 5 (5 min.).

* For expts. 8 and 9 a "granule fraction" of the residue was used.

EXPERIMENTAL DATA

Preliminary experiments were carried out with homogenates of the mouse liver, which formed the principal basis for Ackermann's (1) investigation.

These were prepared in 0.9% KCl-0.01M PO_4 buffer pH 7.3 rather than in the unbuffered isotonic KCl which Ackermann employed, since buffering is a necessary feature of the washed kidney residues which we wished particularly to study. The homogenates were assayed on the basis of oxygen utilization in Ackermann's malonate-inhibited system, and the data are summarized in Table I. It became apparent that, under our experimental conditions, there was no consistent or satisfactory linear relationship between oxygen utiliza-

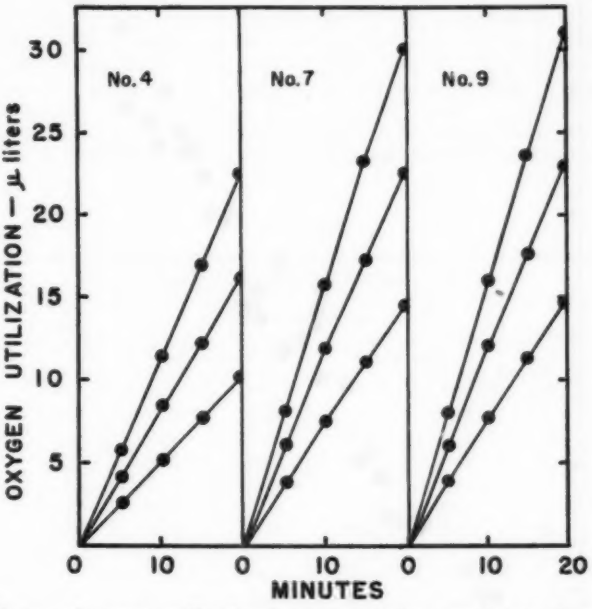

FIG. 3. Course of oxygen utilization in representative experiments from Table VI. Expt. 4—without, expts. 7 and 9—with added cytochrome *c*. Expt. 9—"granule fraction" of residue.

tion and the amount of homogenate present, over the range tested (20 to 60 mg. of tissue per vessel); and furthermore that the oxygen utilization was poorly sustained with the lower levels of tissue concentration.

Since Ackermann's system did not seem to offer a ready solution to our problem, we explored the possibilities of a malonate-inhibited system with washed residues of the rabbit kidney, under conditions similar to those previously reported (5, p. 392). Several representative experiments are given in Table II, and the results obtained indicated the unsuitability of such a system as a basis for an assay procedure. Further exploration of malonate inhibition of α-ketoglutarate oxidation, as a function of substrate/inhibitor ratio (Table

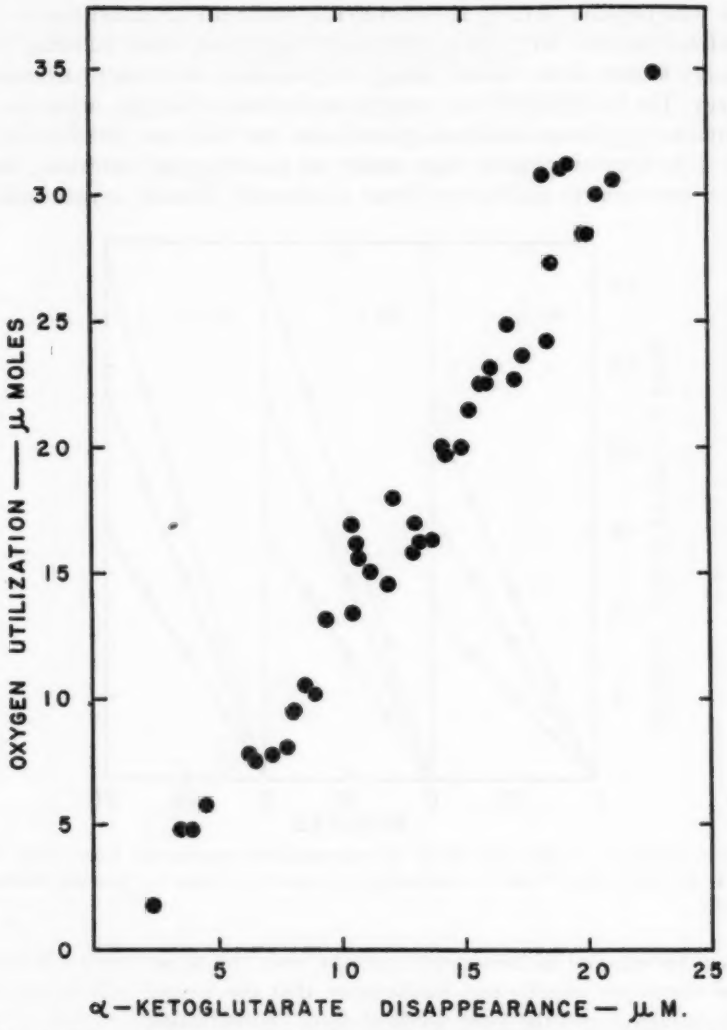

FIG. 4. Chart of all oxygen utilization and α-ketoglutarate disappearance data in Tables V and VI, with the exception of those figures marked with an asterisk in Table V.

III), was similarly discouraging; and we turned to the possibilities of assay in the absence of malonate, with the system operating at maximal or near-maximal levels.

The experimental data are summarized in the accompanying tables and charts, and are largely self-explanatory. Only a few preliminary experiments

TABLE VII

Oxygen Utilization and α-Ketoglutarate Disappearance in the Presence of Varying Concentrations of Cytochrome c, in Experiments with Washed Residues of the Rabbit Kidney

EXPT. NO.	NITROGEN PER VESSEL	ADDED CYTOCHROME c, FINAL CONCENTRATION	α-KETOGLUTARATE				OXYGEN UTILIZATION	
			At 0 min.	At 10 min.	Disappearance			
	mg	*molarity*	*μM*	*μM*	*μM*	*μM/mg N/hr*	*μM*	*μM/mg N/hr*
1	2.62	—	37.4	30.9	6.5	14.9	8.3	19.0
	2.62	0.4×10^{-5}	36.5	28.0	8.5	19.4	10.7	24.5
	2.62	0.7×10^{-5}	36.2	28.2	8.0	18.3	10.6	24.3
	2.62	1.0×10^{-5}	36.0	27.4	8.6	19.7	11.1	25.5
2	2.69	—	34.8	23.7	11.1	24.8	12.5	27.9
	2.69	1.0×10^{-5}	34.5	21.2	13.3	29.7	15.8	35.2
	2.69	2.0×10^{-5}	33.6	20.9	12.7	28.3	15.2	33.9
	2.69	3.0×10^{-5}	34.2	21.4	12.8	28.6	16.0	35.8

α-Ketoglutarate, initial, 42 μM. Each vessel contained 0.6 ml. kidney residue and, where indicated, sufficient 3×10^{-4}M cytochrome *c* to give desired final concentration. Equilibration period 5 min.

TABLE VIII

Oxygen Utilization and α-Ketoglutarate Disappearance in the Presence of Cytochrome c (1×10^{-4}M), Using Varying Amounts of Rabbit Kidney Residues

EXPT. NO.	NITROGEN PER VESSEL	α-KETOGLUTARATE				OXYGEN UTILIZATION	
		At 0 min.	At 10 min.	Disappearance			
	mg	*μM*	*μM*	*μM*	*μM/mg N/hr*	*μM*	*μM/mg N/hr*
1	1.36	38.2	32.2	6.0	26.5	7.4	32.9
	2.04	37.3	27.4	9.9	29.1	11.0	32.3
	2.72	35.4	23.1	12.3	27.1	14.3	31.6
2	1.27	39.6	33.1	6.5	30.7	6.7	31.5
	1.90	38.6	28.3	10.3	32.5	10.0	31.7
	2.54	35.4	23.9	11.5	27.2	13.0	30.8
3	1.37	38.4	32.0	6.4	28.0	7.6	33.3
	2.05	36.3	26.9	9.4	27.5	11.2	32.8
	2.74	34.5	22.1	12.4	27.2	14.4	31.6
4	1.36	39.1	32.0	7.1	31.3	7.7	33.8
	2.04	37.0	26.6	10.4	30.6	11.3	33.2
	2.72	35.5	21.8	13.7	30.2	14.9	32.8
5	1.29	39.5	32.2	7.3	34.0	7.4	34.3
	2.58	35.9	23.3	12.6	29.3	14.7	34.3
6	1.26	39.7	32.8	6.9	32.9	6.9	32.8
	1.26	39.7	32.8	6.9	32.9	7.0	33.4
	2.52	36.2	23.7	12.5	29.8	13.9	33.0
	2.52	35.8	23.1	12.7	30.2	14.0	33.3

α-Ketoglutarate, initial, 42 μM. Each vessel contained 0.3, 0.45 or 0.6 ml. kidney residue and 0.1 ml. 3×10^{-4}M cytochrome *c*. Equilibration period 5 min.

were carried out with blendor homogenates of the kidney (Table IV, Figure 1). In experiments with washed residues of the kidney, the course of oxygen

TABLE IX

Oxygen Utilization and α-Ketoglutarate Disappearance in the Presence of Cytochrome c ($1 \times 10^{-4}M$) and Varying Amounts of DPN and Cocarboxylase, in Experiments with Washed Residues of the Rabbit Kidney

EXPT. NO.	NITROGEN PER VESSEL	DPN	COCARBOXYLASE	α-KETOGLUTARATE				OXYGEN UTILIZATION	
				At 0 min.	At 10 min.	Disappearance			
	mg	*γ/ml*	*γ/ml*	*μM*	*μM*	*μM*	*μM/mg N/hr*	*μM*	*μM/mg N/hr*
1	2.28	—	—	35.6	26.0	9.6	25.3	11.7	30.9
	2.28	16.7	—	36.0	25.8	10.2	26.8	11.7	30.9
Fresh	2.28	—	16.7	36.2	26.1	10.1	26.6	12.0	31.5
	2.28	16.7	16.7	36.2	26.0	10.2	26.8	12.6	33.3
2	2.20	—	—	35.6	26.0	9.6	26.2	11.5	31.4
	2.20	33.3	—	36.6	26.4	10.2	27.8	11.4	31.2
Fresh	2.20	—	33.3	36.4	26.7	9.7	26.4	11.2	30.6
	2.20	33.3	33.3	36.9	26.7	10.2	27.8	11.3	30.9
3A	2.16	—	—	37.2	28.4	8.8	24.4	10.6	29.4
	2.16	66.7	—	37.0	28.2	8.8	24.4	10.3	28.5
Fresh	2.16	—	66.7	37.6	28.6	9.0	25.0	10.4	29.0
	2.16	66.7	66.7	37.6	28.5	9.1	25.3	10.7	29.8
3B	2.16	—	—	40.0	36.2	3.8	10.6	5.0	14.0
	2.16	66.7	—	39.4	35.2	4.2	11.7	5.2	14.4
24 hrs. old	2.16	—	66.7	39.8	36.2	3.6	10.0	4.1	11.4
	2.16	66.7	66.7	39.8	35.6	4.2	11.7	5.2	14.5
4A	2.29	—*	—*	—	—	—	—	8.5	22.2
	2.29	—	—	—	—	—	—	11.9	31.1
Fresh	2.29	66.7	—	—	—	—	—	11.7	30.6
	2.29	—	66.7	—	—	—	—	11.0	28.9
4B	2.29	—*	—*	38.2	32.0	6.2	16.2	6.5	17.1
	2.29	—	—	38.2	29.0	9.2	24.1	9.6	25.3
18 hrs. old	2.29	66.7	—	37.6	28.6	9.0	23.6	10.1	26.5
	2.29	—	66.7	37.8	30.0	7.8	20.4	9.4	24.6

α-Ketoglutarate, initial, 42.6 μM. Each vessel contained 0.5 ml. kidney residue, 0.1 ml. 3×10^{-4}M cytochrome *c* and, where indicated, varying amounts of 0.05% DPN and/or 0.05% cocarboxylase. Equilibration period 5 min.

* No added cytochrome *c*.

utilization and α-ketoglutarate disappearance was followed as a function of time (Table V, Figure 2). A series of exploratory assays was then accomplished with varying amounts of residue, with and without added cytochrome *c*, and for the most part with equilibration and experimental periods of 10 and

20 minutes respectively (Table VI). Oxygen utilization was in general linear throughout the 20-minute period (Figure 3), and a plot of oxygen utilization vs. α-ketoglutarate disappearance showed a reasonably close parallelism between the two over a fairly wide range (Figure 4).

In view of the rather rapid initial rate of α-ketoglutarate disappearance (Figure 2), it was decided to adopt an equilibration period of only 5 minutes as a routine; and the experimental period was reduced from 20 to 10 minutes. Both of these changes were regarded as particularly desirable for work with larger amounts of residue, or with unusually active preparations. The effect of

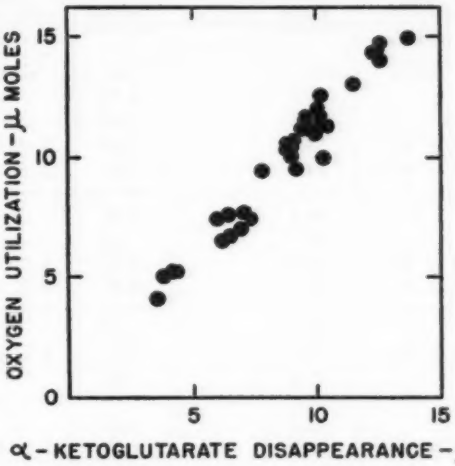

FIG. 5. Chart of all oxygen utilization and α-ketoglutarate disappearance data in Tables VIII and IX. For expt. 6, Table VIII, the average of the duplicate determinations at each of the two nitrogen levels has been charted as a single point.

added cytochrome *c* was re-examined (Table VII), and a final concentration of 1×10^{-5}M was accepted as suitable for present purposes. A second series of assays was accomplished under the revised conditions (Table VIII), with results which would appear to establish the usefulness of the procedures employed. The effect of added DPN and cocarboxylase was explored in a third series of assays (Table IX), and it became apparent that these two factors, alone or in combination, were without significant stimulatory effect upon the system. A plot of oxygen utilization vs. α-ketoglutarate disappearance in the second and third series confirmed the close parallelism between the two (Figure 5).

We were led to examine the possible effectiveness of DPN and cocarboxylase as components in our system by the observations of Ackermann (1), Huennekens and Green (11, 12), Judah (13), Green *et al.* (14), Stumpf *et al.* (15),

etc. Their non-effectiveness under our experimental conditions is evident from the data, and any extended discussion of the problems involved would be beyond the scope of this report.

TABLE X

Oxygen Utilization, with α-Ketoglutarate as Substrate, with and without Added Cytochrome c, in the Presence of Mitochondrial Fractions Prepared from Rabbit Kidney Cortex in Sucrose and in Sucrose-KCl-PO_4 Buffer

FRACTIONS PREPARED IN 0.25M SUCROSE				FRACTIONS PREPARED IN 0.25M SUCROSE–0.9% KCl–0.01M PO_4 BUFFER pH 7.3			
Expt. No.	Nitrogen per vessel	Added cytochrome *c* final concentration	Oxygen utilization	Expt. No.	Nitrogen per vessel	Added cytochrome *c* final concentration	Oxygen utilization
	mg	*molarity*	*μM/mg N/hr*		*mg*	*molarity*	*μM/mg N/hr*
1	1.50	—	33.0	5	1.17	—	33.3
	1.50	—	35.7		1.17	—	35.3
	1.50	1×10^{-5}	33.0		1.17	1×10^{-5}	41.4
	1.50	1×10^{-5}	34.6		1.17	1×10^{-5}	41.7
2	1.34	—	26.1	6	1.47	—	32.9
	1.34	—	25.2		1.47	—	34.2
	1.34	1×10^{-5}	24.8		1.47	1×10^{-5}	37.1
	1.34	1×10^{-5}	25.0		1.47	1×10^{-5}	37.7
3	0.94	—	29.2	7	1.11	—	31.5
	0.94	1×10^{-5}	28.4		1.11	1×10^{-5}	41.9
	1.57	—	25.2		1.86	—	33.3
4	1.04	—	31.7	8	1.25	—	33.4
	1.04	1×10^{-5}	30.1		1.25	1×10^{-5}	39.7
	1.72	—	29.0		2.09	—	33.1
				9	1.16	—	33.5
					1.16	1×10^{-5}	42.7
					1.94	—	35.3

Each vessel contained 42 μM α-ketoglutarate and 0.3 or 0.5 ml. of a suspension of "mitochondria" prepared either strictly according to the method of Schneider (16), in sucrose alone, or by his method modified only by the incorporation of KCl and PO_4 buffer in the sucrose medium. Tissue homogenized in all-glass apparatus of the Memorial type (without beads on pestle), and passed through 0.5 mm. sieve before centrifugation. Equilibration period 5 min. Figures for oxygen calculated from utilization during first 10 min.

The adaptability of our procedure to assay of α-ketoglutaric acid oxidase activity in mitochondrial fractions of kidney was explored in a preliminary way. In terms of oxygen utilization, added cytochrome *c* was without stimulatory effect upon the system when such fractions were prepared in isotonic (0.25M) sucrose, according to the method of Schneider (16); but exerted a marked stimulatory effect when Schneider's method was modified by the

addition of 0.9% KCl and 0.01M PO_4 buffer pH 7.3 to the sucrose medium (Table X). This difference between the two types of preparation was confirmed in initial experiments with α-ketoglutarate disappearance (Table XI).

Our data on mitochondrial fractions, as on homogenates, are admittedly fragmentary. However, it is obvious that, from the standpoint of assay, each type of preparation offers its own particular problems, which must be thoroughly investigated in each case. While the data indicate the usefulness of our assay procedure for α-ketoglutaric acid oxidase activity in saline-washed

TABLE XI

Oxygen Utilization and α-Ketoglutarate Disappearance, with and without Added Cytochrome c, in the Presence of Mitochondrial Fractions Prepared from Rabbit Kidney Cortex in Sucrose and in Sucrose-KCl-PO_4 Buffer

EXPT. NO.	NITROGEN PER VESSEL	ADDED CYTOCHROME *c*, FINAL CONCENTRATION	α-KETOGLUTARATE			OXYGEN UTILIZATION	
			At 0 min.	At 10 min.	Disappearance		
Fractions Prepared in 0.25M Sucrose							
	mg	*molarity*	*μM*	*μM*	*μM*	*μM/mg N/hr*	*μM*	*μM/mg N/hr*
1	1.61	—	39.0	30.4	8.6	32.1	7.1	26.3
	1.61	1×10^{-5}	39.0	30.8	8.2	30.6	7.5	27.9
2	1.32	—	38.0	32.5	5.5	25.0	6.2	28.2
	1.32	1×10^{-5}	38.2	31.7	6.5	29.5	5.8	26.1
Fractions Prepared in 0.25M Sucrose-0.9% KCl-0.01M PO_4 Buffer pH 7.3							
3	1.26	—	39.0	32.9	6.1	29.1	6.4	30.6
	1.26	1×10^{-5}	39.2	31.3	7.9	37.7	8.0	38.0
4	1.41	—	39.6	34.3	5.3	22.6	6.8	29.1
	1.41	1×10^{-5}	39.4	32.0	7.4	31.5	8.3	35.5

α-Ketoglutarate, initial, 42.3 μM. Each vessel contained 0.3 ml. of a suspension of "mitochondria" prepared as described for Table X. Equilibration period 5 min.

kidney residues of the type principally investigated, its applicability to other types of preparation must await further detailed study.

SUMMARY

Satisfactory assay of α-ketoglutaric acid oxidase activity in saline-washed residues prepared from blendor homogenates of the rabbit kidney can be accomplished in the presence of phosphate buffer (pH 7.3) and of added magnesium ions, adenylic acid and cytochrome *c*, with the experimental system operating at maximal or near-maximal rates. There is a sufficiently close parallelism between oxygen utilization and α-ketoglutarate disappearance so that the level of one can be inferred with reasonable accuracy from the level

of the other. Diphosphopyridine nucleotide (DPN) and thiamine pyrophosphate (cocarboxylase), alone or in combination, are without significant stimulatory effect upon the system. Applicability of the assay procedure to other types of preparation (homogenates, mitochondrial fractions, etc.) must await further study.

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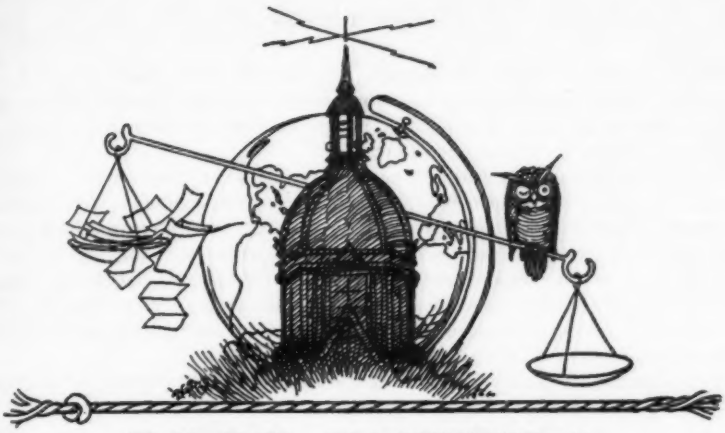

BOOK REVIEWS

(These reviews represent the individual opinions of the reviewers and not necessarily those of the members of the Editorial Board of the Bulletin)

Neurosurgery. An Historical Sketch. (American Lecture Series). By GILBERT HORRAX. 142 pp., $3.75. *Charles C. Thomas, Springfield, Illinois.*

This historical account of the development of neurological surgery is divided into seven time periods: the prehistoric and Egyptian eras; the Greek or Hippocratic and Galenic era; the mediaeval period; the Renaissance; the pre-Listerian period; the pre-Horsley period and the era of neurosurgery as a specialty, 1890 to the present. The author then proceeds to describe the evolution of various neurosurgical operations during each of these periods. This is done very nicely by picking out one or two outstanding surgeons of each period and describing in some detail their surgery and what each considered to be the proper indications for operation.

The author makes free use of direct quotations from the surgeons of each period. This adds greatly to the interest and readability of the book. The ancient trephined skulls and the works of men like Hippocrates, Galen and Paré have been described many times before and it is easy for such an historical account to become dull and uninteresting. The author avoids this by not becoming entangled in details. By the time the reader reaches the period of Horsley he has been given a clear background as to why neurosurgery was ready to develop into a surgical specialty at that time.

One-half of the book is taken up with the development of neurological surgery since 1890. Emphasis is laid on the contribution of Sir Victor Horsley during the period of 1890 to 1910. Although cases of brain tumor were operated upon with increasing frequency, the results were discouraging. The author quotes statistics from Kocher, Ballance, Krause, and others to bear out the forlorn outlook for patients with brain tumors. The author describes how at this point in the development of neurological surgery, if further progress were to be made, it was obvious that someone would have to devote his entire time to perfecting new techniques for operations upon the central nervous system.

It is at this point that the appearance of Harvey Cushing upon the scene of neurological surgery is described. His technical and investigative contributions are emphasized throughout the remainder of the book. The rapid progress made in the treatment of a number of

conditions is related—trigeminal neuralgia, pituitary tumors, intractable pain, acoustic tumors, spinal cord injuries, head injuries, brain abscess, epilepsy, and a number of others. Credit is given to the many neurological surgeons who have contributed to the progress of neurological surgery in the past forty years.

This is a well-told story in which the author ably fulfills the intent set forth in the introduction. "It is incumbent upon us, therefore, to follow the evolution of this and other neurosurgical operations through the centuries; to learn what were the indications for performing these operations from the earliest times down to our own; and finally to review, especially in the modern era, not only the great advances in technic, but also the increasing number of conditions which have come within the neurosurgical sphere, together with the results which have been enormously better with our increasing diagnostic, pathologic, physiologic and technical knowledge."

The author has lived through a considerable part of the development of neurological surgery in its so-called modern era and one realizes that much of what is said in this book is spoken with personal knowledge and authority. There is probably much more the author could tell. The only regret of a young neurological surgeon after reading this excellent book is that it is not longer.

H. C. J.

Fractures, Dislocations and Sprains, 5th edition. By JOHN ALBERT KEY and H. EARLE CONWELL. 1232 pp., $16.00. *The C. V. Mosby Co., St. Louis, Missouri.*

The fifth edition of this book is an up-to-date outgrowth of the original volume. The general form, the clear descriptions, the profuse illustrations and the conservative approach of the authors characterize the new edition as they did the earlier volumes. There have been changes, most of them beneficial. In spite of the addition of new portions, the material has been kept in a single volume of essentially the same size. The publishers, in resetting the book, have made improvements in the type and in the reproduction of the illustrations.

The deletion of the chapters on The Workmen's Compensation Law Affecting Fracture Cases and Medicolegal Aspects of Fracture Cases has been offset by the new material on skeletal injuries. Recent progress in the treatment of compound fractures is introduced by consideration of the factors of shock and the role of the newer antibiotics. Intramedullary nailing is described as a newer method of treatment of fractures of some of the long bones. A new section on lesions of the intervertebral discs gives a better understanding of injuries of the spine.

The book continues "to furnish a practical working guide in the management of fractures, dislocations and sprains" for "the student, the general practitioner and the surgeon."

I. W. N.

Atelektasen der Lunge. By HANNS ALEXANDER. 58 pp., Kartoniert DM 8.70. *Georg Thieme Verlag, Stuttgart, Germany.*

The author of this monograph (a radiologist) has done an excellent job of presenting the subject not only in its radiological aspects but also from the point of view of the physiology and pathology underlying the problem of atelectasis.

He considers the problem of atelectasis under two separate headings. One of these includes the ordinary conception of this pathological process—that is obstruction of a bronchus followed by an absorption of air through the alveoli. The second considers the possibility that atelectasis may occur as a result of active spastic contraction of the bronchi as well as an actual retraction of lung tissue.

The book is illustrated by a great many excellent radiographs. The bibliography is perhaps somewhat small for a subject about which so much has been written.

D. F. P.

Die Hämolytischen Syndrome im Kindesalter. By CONRAD GASSER. 322 pp., Ganzleinen DM 45. *Georg Thieme Verlag, Stuttgart, Germany.*

This is an interesting general introduction to clinical observations in various types of hemolytic anemia. Though no new original observations are recorded the literature relative to the hemolytic syndromes seems well covered.

The first portion of the book is devoted to general aspects of hemolysis. The shape of the erythrocyte is considered as a factor in the erythrocyte destruction. Erythrostasis, mechanical trauma, hemolysins, and phagocytosis are briefly discussed. Certain general symptoms and therapeutic measures are then mentioned.

This is followed by sections devoted to discussions of specific hemolytic entities such as those associated with certain drugs and infections, "morbus haemolyticus neonatorum," congenital hemolytic icterus, paroxysmal nocturnal hemoglobinuria, etc. This reviewer believes certain of the cases of anemia (Jacksch, Haden, Dyke-Young) classified here by eponym are not sufficiently understood to be considered in separate categories.

P. F. W.

The Merck Index of Chemicals and Drugs, 6th edition. 1167 pp., $7.50 (regular edition), $8.00 (thumb-index edition). *Merck & Co. Rahway, N. J.*

Almost everyone who works with chemicals has had occasion to use the Merck Index to find some elusive datum; and such is the organization of this handbook that the specific item can generally be located without difficulty. The Merck Index was first published in 1889 and has now reached its sixth edition. For the information of those unfamiliar with the Index, the main section consists of 1025 pages about chemical compounds and drugs, giving in concise fashion many of their properties. In addition there are a series of appendices containing tables of constants etc., valuable to the laboratory worker in his everyday tasks. Those who have used this volume previously can be assured that most of the newer drugs have been added, and that there have been compiled such new features as a table of standard buffers for calibrating pH measurements and a table of radioactive isotope giving half lives and type of radiation.

The practicing physician may find the Index useful chiefly as a reference for converting popular and proprietary names of drugs into more meaningful terms: this is not to be undervalued in a day when a doctor may be faced with the problem of treating a patient for poisoning with E 605 or K III. Not the least attractive feature of this edition is the professional workmanship of the publisher, including for the benefit of the less meticulous researcher, a cover resistant to water and acid. The cover is also said to resist mildew; an unnecessary precaution in most laboratories, where this handbook will be in constant use.

C. G. Z.

Cybernetics, Transactions of the Eighth Conference, March 15–16, 1951, Edited by HEINZ VON FOERSTER, 240 pp., $4.00. Josiah Macy, Jr. Foundation, New York.

The book contains six papers dealing with communication and information theory which were read before the cybernetics conference, a multi-discipline group made up of physicists, electrical engineers, social scientists, anthropologists, psychologists, neurophysiologists, psychiatrists, and philosophers, who have met yearly since 1946 to consider

problems of "circular, causal and feedback mechanisms in biological and social systems." The papers were brief and were intended to promote discussion. The material included varied from a plan for a maze-solving machine, through considerations of types of communication among animals, the meaning of language, communication among men, to a study of patterns of information transfer in human problem-solving groups.

The discussion that followed each presentation was lively and vigorous, and, as before, constitutes the most interesting part of the publication. In this conference, there was a more marked difficulty in intragroup communication with a division of the group along the line of clinical versus basic science interests. The members consider this problem from time to time, but only in terms of need for clearer language and symbolic representations for the concepts involved. The effect of anxiety and the non-reporting parts of the mind on communication is brought up by those members who are daily concerned with it, but no real concensus is arrived at.

Because of the dependence of a knowledge of previous discussions, the interest of this volume is more limited than previous reports. In the appendix, a glossary of terms used in the information theory is included, and there is a bibliography covering the material of each paper.

A. R. McC.

BOOKS RECEIVED FOR REVIEW, AUGUST 1952

The Collected Papers of Adolf Meyer. Four Volume Set $30.00. Edited by EUNICE WINTERS. Vol. IV, Mental Hygiene. With an introduction by ALEXANDER H. LEIGHTON. 585 pp., *The Johns Hopkins Press*, Baltimore, Md.

Die Nachkommen Geisteskranker Elternpaare. By GÜNTER ELSÄSSER. 340 pp., Ganzleinen DM 33. *Georg Thieme Verlag, Stuttgart, Germany.*

Für und Wider Die Sterilisierung aus Eugenischer Indikation. By HANS NACHTSHEIM. 64 pp., Kart. DM 5.70. *Georg Thieme Verlag, Stuttgart, Germany.*

Grundlagen und Sprache der Phthise- und Tuberkuloseforechung. By SIEGFRIED GRÄFF. 61 pp., Kart. DM 6.60. *Georg Thieme Verlag, Stuttagrt, Germany.*

Hautplastiken. Indikation and Technik. By H. I. ZÜRICH. 136 pp., Ganzl. DM 22.50. *Georg Thieme Verlag, Stuttgart, Germany.*

Proceedings of the Forum Sessions; Thirty-Seventh Clinical Congress of the American College of Surgeons, San Francisco, California, November, 1951. Surgical Forum Committee; Owen H. Wangensteen, Warren H. Cole, Robert E. Gross, Michael L. Mason, Carl A. Moyer and I. S. Ravdin. 667 pp., $10.00. *W. B. Saunders Co., Philadelphia, Pa.*

The Story of the Adaptation Syndrome. By HANS SELYE. 225 pp., $4.50. *Acta Inc. Montreal, Quebec, Canada.*

A Textbook of Pharmacology. Principles and Application of Pharmacology to the Practice of Medicine. By WILLIAM T. SALTER. 1240 pp., $15.00. *W. B. Saunders Co., Philadelphia, Pa.*

The Treatment of Acute Dehydration in Infants. By a Working Team appointed and advised by the Committee on Acute Infections in Infancy. Medical Research Council Memorandum No. 26. 49 pp., 3s. net. *Her Majesty's Stationery Office, London, England.*

Über den Wahn. By KURT SCHNEIDER. 48 pp., Kart. DM 3.90. *Georg Thieme Verlag, Stuttgart, Germany.*